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CROISSANCE PONDERALE ET DUREE D'INTERMUE CHEZ *CARAUSIUS MOROSUS*, Br.

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L'étude quantitative de la croissance d'un Phasmide, *Carausius morosus* Brunner, m'a conduite à étudier le taux d'accroissement pondéral d'une mue à la suivante, et la durée d'intermue; l'ensemble de ces résultats permet de caractériser du point de vue biométrique un lot d'animaux élevés au laboratoire, dans des conditions contrôlées.

Mes observations ont porté sur un échantillon provenant d'un élevage de plusieurs milliers de Phasmes constitué de la manière suivante:

Soixante treize jeunes, éclos à la même date, furent élevés dans une même enceinte jusqu'à la troisième mue; à ce moment ils constituent un lot aussi homogène que possible, et atteignent une taille suffisante pour rendre les mensurations aisées. Chaque animal fut donc isolé au moment de sa troisième mue et maintenu, dès lors, en cage individuelle numérotée, dans une étuve humide à 22°, et régulièrement approvisionné en lierre frais. Dans ces conditions il a parcouru ainsi les 4è, 5è et 6è intermues en subissant les 3è, 4è, 5è et 6è mues (m_3, m_4, m_5, m_6). Les durées des intermues sont évaluées et les poids (p_3, p_4, p_5, p_6) sont déterminés à jeun, au mg près, dans les douze heures suivant la mue. Les taux d'accroissement calculés individuellement sont:

$$\frac{p_4}{p_3} = r_3, \frac{p_5}{p_4} = r_4, \frac{p_6}{p_5} = r_5.$$

Les données numériques dont il a été tenu compte dans les calculs et graphiques ne concernent que 58 animaux; en effet j'ai éliminé 14 individus dont l'une des mues n'avait pu être rigoureusement repérée au cours des 66 jours d'élevage individuel.

I—En ce qui concerne les taux d'accroissement pondéral, ces

données numériques traitées par la méthode statistique élémentaire fournissent les grandeurs consignées dans le tableau I:

TABLEAU I
CROISSANCE PONDÉRALE DE CARAUSIUS MOROSUS ENTRE LA 3È ET LA 6È MUE

	Valeurs moyennes: $\bar{r}$	Ecart type: σ	Coefficient de variabilité $100\ \sigma/\bar{r}$
$\bar{r}_3$	2,042 ± 0,028	0,212	10,3
$\bar{r}_4$	2,074 ± 0,036	0,274	13,2
$\bar{r}_5$	1,870 ± 0,033	0,250	13,4
$\bar{r}_4 - \bar{r}_3$	0,032 ± 0,055	0,417	

Ces grandeurs indiquent en particulier que:

1° / Pour l'ensemble du lot, les valeurs moyennes des taux d'accroissement $\bar{r}_3$, et $\bar{r}_4$ peuvent être considérées comme égales à 2; le calcul montre que la différence $\bar{r}_4 - \bar{r}_3$ n'est pas significative. Par contre la mue imaginale est caractérisée par un taux $\bar{r}_5 < 2$. Les résultats sont en accord avec ceux de Teissier (1931).

2° / Malgré les conditions favorables d'homogénéité du lot d'animaux la variabilité est assez forte.

3° / Les taux d'accroissement r_3, et r_4, relatifs à deux mues consécutives chez un même animal, ne sont pas indépendants. Le calcul d'après les chiffres obtenus et en utilisant l'identité:

$$\sigma^2_{(r_4 - r_3)} \equiv \sigma^2_{r_3} + \sigma^2_{r_4} - 2\sigma_{r_3}\sigma_{r_4}\rho$$

donne $\rho = -0{,}463$, valeur tout à fait significative.

Le coefficient de corrélation est l'expression mathématique du degré de liaison des taux d'accroissement successifs. Cette dépendance est ici une sorte de compensation: un accroissement particulièrement fort au cours d'un stade donné est suivi d'un accroissement particulièrement faible au stade suivant. Au contraire, Teissier (1937), utilisant des chiffres relatifs à la croissance linéaire des Crustacés Décapodes, pouvait conclure à l'indépendance des taux d'accroissement successifs.

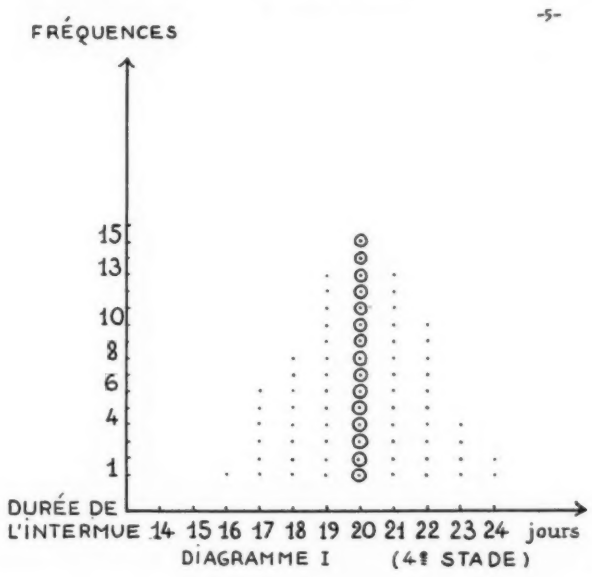
La divergence entre les résultats obtenus par Teissier chez les Décapodes et mes propres résultats s'explique vraisemblablement par la différence de constitution du lot d'animaux observés: échantillons prélevés au hasard dans une population naturelle pour les résultats donnés par Teissier, et, pour les miens, choix, dans un élevage homogène d'animaux parthénogénétiques, d'oeufs susceptibles de donner

des animaux aussi semblables que possible, suivi d'un élevage individuel dans des conditions contrôlées. On est en droit de penser que, dans de telles conditions, la variabilité analysée tient essentiellement à l'action fortuite des facteurs internes; un phénomène compensateur liant les taux d'accroissement de deux mues consécutives chez un même individu est évidemment dû à des facteurs internes; dans le cas présent il est donc naturel qu'il se traduise nettement par un coefficient de corrélation significatif.

II—En ce qui concerne les durées d'intermue individuelles observées je donne successivement les trois diagrammes de fréquence correspondant aux 4è, 5è et 6è intermues. Leur examen montre que:

1° / Mes résultats sont en accord avec les durées moyennes observées par Pflugfelder (1937).

2° / Les animaux ayant présenté une durée d'intermue coïncidant avec le mode du diagramme I et qui sont représentés par des points cerclés se répartissent au hasard sur les diagrammes II et III. Contrairement aux taux successifs d'accroissement pondéral les durées d'intermues consécutives, chez un même individu, sont indépendantes.



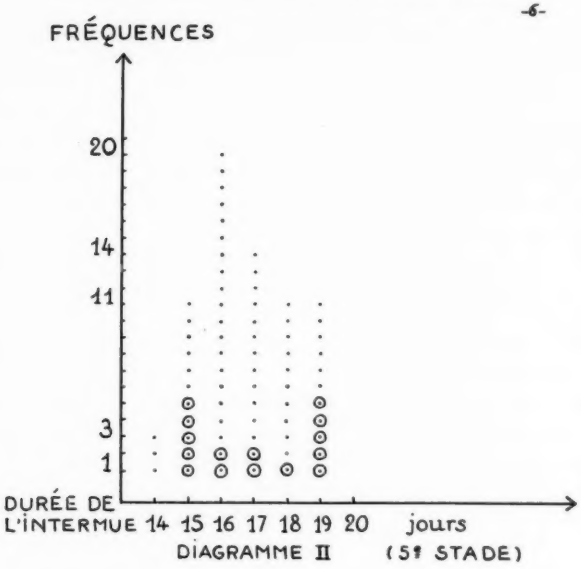


DIAGRAMME II (5e STADE)

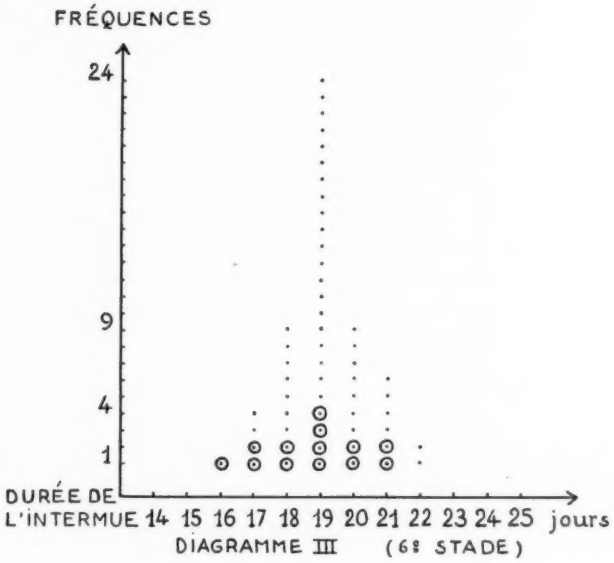


DIAGRAMME III (6e STADE)

Summary

In a quantitative study of growth of a Phasmid, *Carausius morosus Brunner*, the rate of weight-increase from one moult to the succeeding one and the interval between two ecdyses were recorded. Measurements have been performed with a homogeneous lot of about fifty animals reared under the controlled conditions described, from eggs hatched the same day.

Calculations and diagrams show that in a given insect:

1° / There is a negative correlation between two consecutive rates of weight-increase;

2° / There is no correlation between the durations of two consecutive instars.

On the other hand it is noted that Teissier (1937) did not find a correlation between the rates of length-increase in Decapods; and it is suggested that the difference between Teissier's results and my own is due to different techniques of sampling.

References

1. Pflugfelder, O. 1937. Bau, Entwicklung und Funktion der Corpora Allata und Cardiaca von *Dixippus morosus* (Br). *Zeit. Wiss. Zool.*, **149**, 477-512.
2. Teissier, G. 1931. Recherches morphologiques et physiologiques sur la croissance des Insectes. *Trav. Stat. Biol. Roscoff*, **9**, 29-238. Paris.
3. ———. 1937. Sur la variabilité du taux d'accroissement lors de la mue des Crustacés Décapodes. Soixante dixième Congrès des Sociétés Savantes. 303-306. Paris.

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THE MITOSIS STIMULATING EFFECTS OF FRESH ADULT CHICKEN HEART EXTRACT AND OF CHICK EMBRYONIC EXTRACTS*

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It is still generally believed, that of all tissue extracts only those prepared from embryonic tissues, and to a far smaller extent of neoplastic tissues or of certain mitotically active adult tissues such as bone-marrow, are capable of markedly accelerating the rate of growth of cell colonies cultivated in vitro.

However, studies undertaken in this laboratory in recent years (Hoffman and Doljanski, 1939, and Hoffman, Tenenbaum and Doljanski, 1940) have demonstrated that extracts of a number of adult chicken tissues greatly increase the rate of growth of pure colonies of fibroblasts cultivated in vitro in Carrel flasks. Some of these extracts were even more potent in this respect than chick embryonic extract.

These comparative studies on the growth promoting power of adult and embryonic tissue extracts were made using the surface area as an index of colony growth. Since the growth of a cell colony in vitro is a complex phenomenon, involving both the division and the migration of cells, it seemed of interest to complete the work by studying the effect of growth-activating adult and embryonic tissue extracts on the mitotic activity of cultures.

EXPERIMENTAL

Culture Technique

The experiments were performed with third passage cultures of fibroblasts derived from 8-day chick embryo hearts. Each cell colony

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was cut as exactly as possible into two equal parts, each of which was planted on a coverslip in a clot composed of 1 drop of adult chicken plasma and 1 drop of the test medium. Usually one-half was cultivated in embryonic extract and the other in adult chicken heart extract. In some cases the growth of cultures in the plasma-tissue extract medium was compared with that in a plasma-Tyrode's solution medium.

The pairs of cultures thus obtained were treated as hanging drop cultures, and incubated for 24 to 48 hours. They were then fixed in Carnoy's fluid and stained with Giemsa's stain. The total number of mitoses in the zone of growth of each culture was counted, and at the same time the distribution of the various mitotic phases was determined.

Test Media

Fresh adult chicken heart extract was prepared under sterile conditions according to our standard procedure (Hoffman and Doljanski, 1939), by mincing adult chicken hearts with scissors or a Latapie apparatus, and extracting the mash with 4 volumes of Tyrode's solution for 24 hours. The minced tissue was centrifuged and the clear supernatant fluid constituted the heart extract.

Embryonic extracts. Two different types of chick embryo extract were used in this experiment, and are designated below as (a) and (b).

(a) An embryonic extract which was prepared exactly in the same way as the heart extract described above. Eight-day old chick embryos were passed through the embryo extract press and extracted for 24 hours at 6° C. with 4 volumes of Tyrode's solution. The ground tissue was centrifuged at 3500 RPM for 10 minutes and the supernatant fluid constituted the embryonic extract.

This embryonic extract and the heart extract thus represented comparable concentrations of embryonic and adult heart tissues respectively.

(b) The 2nd centrifugate of embryonic tissue (Fischer, 1930, pp. 49) was obtained as follows:

Eight-day old chick embryos were passed through an embryonic extract press, and the pulp was centrifuged at 3500 RPM for 15 minutes. The supernatant fluid (usually constituting no more than 1/3 of the total volume of the embryo pulp) was pipetted off, and Tyrode's

solution was added to restore the original volume. The pulp was well mixed, extracted for 10 minutes at 6° C., centrifuged as before, and the resulting supernatant fluid (2nd centrifugate) was used as embryonic extract.

This extract obviously represents a much higher concentration of embryonic tissue and is much more viscous than the extract (a) described above.

Prolonged experience in our laboratory has shown that this is the most stimulating chick embryonic extract obtainable for use in passage cultures of embryonic chick heart fibroblasts in hanging drops.

Results

1. *Comparison of chick embryo and adult chicken heart extracts prepared in the same manner.*

The embryonic extract used in this experiment was that designated (a) above, representing the same concentration of 8-day chick embryo as the heart extract of heart tissue.

The results, presented in Table 1, indicated that the cultures in heart extract contain considerably more mitoses after 48 hours of cultivation than do the sister cultures in embryonic extract.

TABLE 1

The Total Number of Mitoses in 48 Hour Old Fibroblast Colonies (Sister Halves) Growing in the Presence of Similar Concentrations of Embryonic Extract (A) and Fresh Adult Chicken Heart Extract (B)

No. of Cultures	A.	B.
26725/6	96	331
26727/8	259	584
26729/30	244	456
26731/2	153	335
26733/4	127	387
26735/6	125	532
26737/8	80	219
26739/40	139	554
26741/2	118	421
26747/8	91	411
26749/50	106	112
26888/9	317	347
26900/1	192	311
26902/3	195	282
Average	160.1	377.2

2. *Comparison of optimal concentrations of chick embryo and adult chicken heart extracts.*

The stimulation of mitosis by the "2nd centrifugate" of chick embryos was compared to that of adult heart extract. The results, presented in Table 2, show that the total numbers of mitoses after 24 (Table 2A) and 48 hours of cultivation (Table 2B) are approximately the same in cultures grown in embryonic extract and in heart extract.

It was noted microscopically that the cultures prepared with heart extract were invariably rather thinner than those grown in embryonic extract, the cells in the zone of growth often forming a single layer, and generally covering a larger area than the sister cultures in embryonic extract. In all other respects the two series of cultures appeared identical, and the cells of both were morphologically normal.

Two small control series of sister cultures were made, comparing the number of mitoses observed after 48 hours of incubation when the cultures are grown (a) in a plasma-Tyrode's solution medium and in a plasma-embryonic extract medium (Table 3A), and (b) in a plasma-Tyrode's solution medium as compared with a plasma-heart extract medium (Table 3B). The results show that the cultures grown in embryonic extract or in heart extract contain many times more mitoses than do the sister cultures grown in the presence of Tyrode's solution.

3. *Percentage distribution of mitotic phases in cultures grown in the presence of an optimal concentration of chick embryo extract and of adult chicken heart extract.*

The mitoses observed in cultures grown in media containing optimal concentrations of embryonic and adult heart extract were classed in the usual prophase, metaphase, anaphase, telophase and reconstruction phases. The percentage distribution of these phases was calculated and the results are presented in Table 4A and Table 4B. The tables clearly indicate that after 24 and 48 hours of incubation there is no significant difference in the percentage distribution of mitotic phases between cultures stimulated by optimal concentrations of chick embryo extract or of adult chicken heart extract.

TABLE 2

A. The Total Number of Mitoses in 24 Hour Old Fibroblast Colonies (Sister Halves), Growing in the Presence of an Optimal Concentration of Embryonic Extract (A), and of Fresh Adult Chicken Heart Extract (B)

No. of cultures	A.	B.
E 1/2	284	396
E 11/2	124	211
E 13/4	406	491
E 17/8	218	371
E 19/20	464	565
E 21/2	560	581
E 23/4	580	689
E 51/2	211	226
E 53/4	154	142
E 55/6	255	131
E 57/8	111	130
E 59/60	133	159
E 61/2	116	102
E 63/4	184	160
E 65/6	182	166
Average	265.4	301.3

B. The Total Number of Mitoses in 48 Hour Old Fibroblast Colonies (Sister Halves), Growing in the Presence of an Optimal Concentration of Embryonic Extract (A), and of Fresh Adult Chicken Heart Extract (B)

No. of cultures	A.	B.
26459/60	372	590
26463/4	353	488
26465/6	556	457
26467/8	462	327
26469/70	514	421
26473/4	513	427
26910/1	432	242
26912/3	334	266
26914/5	595	504
26916/7	241	179
26918/9	215	490
E 7/8	436	249
E 9/10	264	219
E 27/8	242	398
E 29/30	486	194
E 31/2	293	203
E 33/4	264	346
E 35/6	138	162
E 69/70	250	170
E 71/2	168	164
E 73/4	172	96
E 77/8	261	263
Average	343.6	352.9

TABLE 3

A. The Total Number of Mitoses in 48 Hour Old Fibroblast Colonies (Sister Halves), Growing in a Plasma-Tyrode's Solution Medium (A), and in a Plasma-Embryonic Extract Medium (B)

No. of cultures	A.	B.
E 37/8	32	536
E 39/40	16	478
E 41/2	13	323
E 81/2	27	204
E 83/4	19	304
Average	21.5	369.0

B. The Total Number of Mitoses in 48 Hour Old Fibroblast Colonies (Sister Halves), Growing in a Plasma-Tyrode's Solution Medium (A), and in a Plasma-Fresh Adult Chicken Heart Extract Medium (B)

No. of cultures	A.	B.
E 43/4	21	301
E 45/6	29	472
E 47/8	40	402
E 49/50	11	441
E 85/6	23	283
E 87/8	21	235
E 89/90	3	362
E 91/2	15	276
Average	20.3	321.4

TABLE 4

A. The Percentage Distribution of Mitotic Phases of 24 Hour Old Fibroblast Colonies (Sister Halves), Growing in the Presence of an Optimal Concentration of Embryonic Extract (A), and of Fresh Adult Chicken Heart Extract (B)

	A. Percentage of					B. Percentage of				
No. of cultures	Pro-phases	Meta-phases	Ana-phases	Telo-phases	Recon-struc-tion phase	Pro-phases	Meta-phases	Ana-phases	Telo-phases	Recon-struc-tion phase
E 1/2	31	25	7	15	22	31	35	4	6	24
E 11/12	30	34	3	5	28	32	30	9	9	20
E 13/14	28	31	5	10	26	30	31	6	9	24
E 17/18	27	31	4	10	28	30	33	5	9	23
E 19/20	28	30	5	10	27	29	33	4	10	24
E 21/22	27	31	4	10	28	29	29	5	12	25
E 23/24	29	31	6	8	26	31	32	5	7	25
E 51/52	24	26	8	9	33	29	29	6	8	28
E 55/56	23	31	10	12	24	22	31	11	10	26
E 57/58	23	35	13	6	23	21	32	20	7	20
E 59/60	23	32	10	9	26	23	37	6	8	26
E 61/62	30	38	8	7	17	24	35	8	8	25
E 63/64	26	29	13	9	23	31	31	10	9	19
E 65/66	19	35	13	6	27	27	34	6	6	27
Average	26.3	31.3	7.8	9.0	25.6	27.8	32.3	7.5	8.4	24.0

TABLE 4 (*continued*)

B. The Percentage Distribution of Mitotic Phases of 48 Hour Old Fibroblast Colonies (Sister Halves), Growing in the Presence of an Optimal Concentration of Embryonic Extract (A), and of Fresh Adult Chicken Heart Extract (B)

	A. Percentage of					B. Percentage of				
No. of cultures	Pro-phases	Meta-phases	Ana-phases	Telo-phases	Recon-struc-tion phase	Pro-phases	Meta-phases	Ana-phases	Telo-phases	Recon-struc-tion phase
26916/7	32	27	6	15	20	26	36	9	10	19
E 7/8	28	31	5	8	28	29	32	5	8	26
E 9/10	28	30	6	11	25	30	36	3	6	25
E 27/28	29	33	4	6	28	26	32	5	11	26
E 29/30	30	32	3	8	27	29	36	5	8	22
E 31/32	24	27	9	14	26	30	32	2	6	30
E 33/34	32	30	5	9	24	28	33	5	10	24
E 35/36	21	34	7	6	32	32	33	2	5	29
E 69/70	20	36	10	10	24	24	34	5	12	25
E 71/72	17	35	9	9	30	24	28	5	19	24
E 73/74	11	35	15	18	21	29	34	4	10	23
E 77/78	20	29	9	15	27	20	31	4	9	36
Average	24.3	31.6	7.3	10 7	26.0	27.1	33.1	4.5	9.5	25.7

Discussion

The results presented in this paper show that optimal concentrations of chick embryonic extract and adult chicken heart extract have approximately the same mitosis-stimulating effects in hanging drop cultures of fibroblasts. However, an "optimal" embryonic extract represents a much greater concentration of embryonic tissue than the heart extract of adult heart tissue, and when the embryonic and heart extracts were prepared in similar concentrations the mitosis-stimulating effect of the heart extract was found to be greater than that of the embryonic extract. It thus seems that per unit weight adult chicken heart tissue stimulates more mitoses than does chick embryonic tissue under the conditions of the experiment.

Relatively few investigators have studied the effects of adult tissue extracts on the growth of tissue cultures.

As early as 1913 Carrel (1913) demonstrated that extracts of a variety of adult tissues stimulated the growth of fibroblasts in vitro, the degree of stimulation varying considerably with the kind of tissue used. The results were confirmed by Walton (1914) and again by Carrel and Ebeling (1923). Carrel, however, was of the opinion that the *permanent* cultivation of cells is possible only when embryonic

extract is present in the medium. It should be noted that all his attempts at permanent cultivation were made with hanging drop cultures only.

Hoffman and Doljanski (1939) and Hoffman, Tenenbaum and Doljanski (1940) using more exact quantitative techniques have shown that the growth promoting potency of tissue extracts is in no way correlated with the state of mitotic activity of the tissue, from which they were made. The extracts of some adult tissues, such as brain and heart muscle which are quite stable mitotically, stimulate the growth of fibroblasts cultivated in Carrel flasks even more than do chick embryonic extracts. On the other hand extracts of adult chicken bone marrow are considerably less active than embryonic extracts. Doljanski, Hoffman and Tenenbaum (1944) found that: "Extracts of tumors affect the rate of cell growth in vitro in essentially the same manner as extracts of normal tissues. In the cases where normal tissue extract stimulated cell growth, tumor extracts acted likewise; in the cases where extracts of normal tissues either did not appreciably affect cell proliferation or inhibited it, tumor extracts inhibited the growth of cultures. The growth promoting power of those tumor extracts which stimulate cell growth is never exceptionally high; it falls in the range of activity of extract of adult heart."

Moreover, Doljanski and Hoffman (1943) have maintained fibroblasts under continuous cultivation with rapid growth in Carrel flasks through numerous passages over a period of one year using adult chicken heart extract as the sole growth stimulant, thus demonstrating that it is possible to cultivate fibroblast colonies indefinitely in the total absence of embryonic extract. The growth rate throughout the entire period of cultivation remained undiminished, and at the end the cells appeared normal, the texture of the colonies being dense and uniform. At the same time these authors reported their failure to grow the same fibroblast colonies indefinitely in hanging drop cultures employing heart extract, thus confirming the experimental results of Carrel (1923).

Trowell and Willmer (1939) determined the mitotic indices of colonies of periostial fibroblasts grown in the presence of various adult tissue extracts, and concluded that a number of these extracts possessed marked growth-promoting properties.

Thus the available data lead to the following conclusions:

1. Adult and embryonic tissue extracts stimulate the growth of cell colonies in vitro in qualitatively identical manners, as regards the area covered by the cultures and the mitotic activity of the cultures.

2. Extracts of different tissues possess different degrees of mitosis-stimulating potency, and these do not depend on the mitotic activity of the tissue in vivo, or on whether the tissue is a new growth.

3. In some cases, and under given experimental conditions, certain adult tissue extracts are even more active than embryonic extracts, both as regards the area covered by the cultures and the mitotic activity of the cultures.

These conclusions are further supported by the fact that in the hands of different investigators all attempts to separate the factors responsible for the in vitro cell growth promoting activity of tissue extracts have so far yielded practically identical results with adult tissue extracts and with embryonic extracts.

(For data on the dialysis of adult tissue extracts see Margoliash, Tenenbaum and Doljanski, 1948; for data on the dialysis of embryonic extracts see Baker and Carrel, 1926, Jacoby, 1937, Tazima, 1940, Fischer and Astrup, 1943; for data on the precipitation of adult tissue extracts with acetone see Margoliash (in press); for data on the precipitation of embryonic extracts with dehydrating organic solvents see Baker and Carrel, 1926, Fischer, 1930, pp. 22).

Summary

The effect of fresh chicken heart extract on the mitotic acitivity of pure cultures of fibroblasts deriving from embryonic chick hearts, was compared to that of chick embryonic extract, in hanging drop cultures, by counting the total number of mitoses in the zone of growth of the cultures.

It was found that:

1. When the embryonic extract and the heart extract are prepared in the same manner so that both represent the same concentration of tissue, the adult chicken heart extract is considerably more active than the embryonic extract.

2. Optimal concentrations of embryonic extract have approximately the same mitosis stimulating effect as the adult chicken heart extract.

3. There is no difference in the percentage distribution of mitotic

phases between the cultures growing in the presence of embryonic extract and those growing in the heart extract.

References

1. Baker, L. E., & Carrel, A. 1926. Action on fibroblasts of the protein fraction of embryonic tissue extracts. *J. Exper. Med.*, **44**, 387-395.

2. ———. 1926. The effect of the amino-acids and dialyzable constituents of embryonic tissue juice on the growth of fibroblasts. *J. Exper. Med.*, **44**, 397-407.

3. Carrel, A. 1913. Artificial activation of the growth in vitro of connective tissue. *J. Exper. Med.*, **17**, 14-19.

4. Carrel, A., & Ebeling, A. K. 1923. Action on fibroblasts of extracts of homologous and heterologous tissues *J. Exper. Med.*, **38**, 499-511.

5. Doljanski, L., & Hoffman, R. S. 1943. The growth activating effect of extracts of adult tissue on fibroblast colonies in vitro. III. The cultivation for prolonged periods. *Growth*, **7**, 67-72.

6. Doljanski, L., Hoffman, R. S., & Tenenbaum, E. 1944. The effect of tumor extracts on the growth of cells in vitro. *Growth*, **8**, 13-31.

7. Fischer, A. 1930. Gewebezuechtung. Muenchen, Mueller and Steinecke.

8. Fischer, A., & Astrup, T. 1943. Untersuchungen ueber die chemischen Eigenschaften des tierischen Wuchsstoffes aus Embryonen. *Pflügers Arch f. d. ges. Physiol.*, **247**, 34-52.

9. Hoffman, R. S., & Doljanski, L. 1939. The growth activating effect of extracts of adult tissue on fibroblast colonies in vitro. I. Experiments with chicken heart extracts. *Growth*, **3**, 61-71.

10. Hoffman, R. S., Tenenbaum, E., & Doljanski, L. 1940. The growth activating effect of extracts of adult tissue on fibroblasts colonies in vitro. II. Experiments with extracts of brain, bone-marrow, liver, lung, smooth muscle, pancreas, kidney, ovary, oviduct, spleen and testis. *Growth*, **4**, 207-221.

11. Jacoby, F. 1937. Migratory and mitotic activity of chick fibroblasts under the influence of dialysate of embryo juice. *Arch. f. exper. Zellforsch.*, **19**, 241-254.

12. Margoliash, E. Studies on the growth promoting factor of adult tissue extracts. VI. The effect of acetone precipitation. *Growth* (in press).

13. Margoliash, E., Tenenbaum, E., & Doljanski, L. 1948. Studies on the growth promoting factor of adult tissue extracts. V. The effect of dialysis. *Growth*, **12**, 1-13.

14. Tazima, M. 1940. Influence of dialysis of the embryonic tissue extract on the growth of tissue in vitro. *Tohoku, J Exper. Med.*, **38**, 8-13.

15. TROWELL, O. A., & WILLMER, E. N. 1939. Studies on the growth of tissues in vitro. VI. The effects of some tissue extracts on the growth of periosteal fibroblasts. *J. Exper. Med.*, **16**, 60-70.

16. WALTON, A. J. 1914. The effect of various tissue extracts upon the growth of adult mammalian cells in vitro. *J. Exper. Med.*, **20**, 554-572.

Growth, 1950, **14**, 19-30.

STUDIES ON THE GROWTH-PROMOTING FACTOR OF ADULT TISSUE EXTRACTS
VI. THE EFFECT OF ACETONE PRECIPITATION*

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The present paper deals with the in vitro growth-promoting ability of acetone fractions of adult chicken heart extract.

Following the demonstration that dialysis of the growth-promoting extract causes a reversible reduction of its activity (Margoliash, Tenenbaum and Doljanski, 1948), we wished to determine whether a similar effect could be obtained by separating the proteins of the extract by means of dehydrating organic solvents as well as by dialysis. Acetone was chosen since total protein precipitation with acetone requires the use of far smaller volumes than with other precipitants such as ethyl alcohol, leading to greater ease in sterile manipulation. Moreover, it is possible to obtain the protein precipitate in an entirely undenaturated form by working below —10° C.

METHOD AND MATERIALS

The tests of growth-promoting power were performed on 3rd passage cultures of fibroblasts derived from 8 day chick embryo hearts. The techniques of cultivation and of growth measurement were those used in the first paper of this series (Doljanski and Doljanski, 1944).

The heart extract used throughout was standard Tyrode's solution extract of acetone-desiccated adult chicken hearts, prepared according to a previously published procedure (Margoliash, 1947).

EXPERIMENTAL

1. *Preparation of the acetone precipitate.*

Five mls. of heart extract of 0° C. were added to 15 mls. of acetone at 0° C. After 5 minutes the rose-colored precipitate was centrifuged

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off at 3500 R.P.M. for 5 minutes. The supernatant fluid was removed to be used in the preparation of the acetone soluble fraction. The tube containing the precipitate was left for 24 hours over H_2SO_4 in vacuo to remove traces of acetone and water. The dry crust of precipitate was broken up with a glass rod, and 5 mls. of sterile Tyrode's solution were added and well mixed with the precipitate. The mixture was extracted for 24 hours at 0° C., with occasional shaking. Only a fraction of the precipitate redissolved under these conditions. The remainder was centrifuged off, and the clear, reddish supernatant fluid was designated as the "acetone precipitate".

The entire procedure was carried out with strict sterile precautions.

2. *Preparation of undenatured acetone precipitate.*

The procedure was exactly as above, except that the temperature of the solution was maintained below —10° C. until the acetone precipitate was entirely free of water, with the help of a mixture of solid CO_2 in alcohol. To ensure desiccation, the precipitate was washed twice with 10 mls. of cold absolute acetone after the original precipitation, and then twice with 10 mls. of cold dry ether.

Under these conditions the precipitate was a very light, fine, reddish powder completely soluble in Tyrode's solution. The solution thus obtained (5 mls. of redissolved precipitate from 5 mls. of heart extract) may be considered to contain the same concentration of acetone-precipitate substances as the original heart extract.

3. *Preparation of the acetone soluble fraction.*

The supernatant fluid from the original acetone precipitate (about 19.5 mls.) was added to 60 mls. of ether. The aqueous layer formed was removed with a separatory funnel and was extracted twice more with 20 mls. of ether each time. The volume of the final aqueous fraction varied from 4.0 to 4.6 mls. It was evaporated to dryness in vacuo over H_2SO_4 for 24 to 48 hours. The light yellow residue was redissolved to the original volume of the aqueous fraction in distilled water and the solution was finally sterilized by passing through a Seitz filter, not less than 15 mls. being filtered at one time.

This solution, light yellow in color, may be considered to contain the same concentration of acetone-soluble substances as the original heart extract. It was designated the "acetone soluble fraction".

4. *Fractional precipitation of heart extract with acetone.*

Under the same conditions employed in the preparation of the total acetone precipitate, 2 mls. of acetone at 0° C. was added to 5 mls. of heart extract diluted with 15 mls. of Tyrode's solution at 0° C. The precipitate was centrifuged off and the concentration of acetone in the supernatant fluid was increased by successive steps to 23, 33, 41, 47, 60 and 75%. With each addition of acetone the precipitate formed was removed, dried and resuspended in 5 mls. of Tyrode's solution as described above for the total precipitate. The entire procedure was carried out under sterile conditions.

In order to determine roughly the relative amounts of precipitate obtained at the various concentrations of acetone a parallel experiment was carried out on a second 5 ml. aliquot of the same sample of heart extract diluted with 15 mls. of Tyrode's solution. Each precipitate was separated, washed twice with 5 mls. of a solution of acetone diluted with distilled water to the concentration in which it was used for the particular precipitation in question, and was then dried and weighed. The percentage of the total weight of acetone-precipitable material contained in each precipitate was calculated.

Relatively little precipitate was formed at 9, 23, 47, 60 and 75%. The main bulk of the material precipitated at 33 and 41% of acetone. All the precipitates, and especially the more bulky ones, had the same rose color as the total acetone precipitate produced with three volumes of acetone.

Results

1. *The acetone precipitate contained somewhat less than 25% of the growth-promoting activity of the original heart extract.*

Had the acetone caused complete precipitation of the active material in the heart extract, the precipitate as prepared above would have had the same growth-promoting effect as the original heart extract. However, a comparison of the growth-promoting activity of the acetone precipitate with that of 50% and 25% dilutions, of the original heart extract in Tyrode's solution, and with that of Tyrode's solution alone (Table 1), clearly shows that the precipitate is slightly less active than 25% heart extract. Evidence will be presented below that this partial recovery of activity in the precipitate is not due to the partial denaturation of protein which occurs during precipitation.

TABLE 1

A. THE AREA IN MM^2 OF 6 DAY OLD FIBROBLAST COLONIES (SISTER HALVES) GROWING IN THE PRESENCE OF 50% EXTRACT FROM ACETONE DESICCATED HEARTS (A), AND OF THE ACETONE PRECIPITATE OF EXTRACT FROM ACETONE DESICCATED HEARTS (B).

No. of cultures		A.	B.
13643/4	a.	65	28
	b.	50	28
13645/6	a.	45	31
	b.	54	28
13753/4	a.	38	10
	b.	36	10
13755/6	a.	54	44
13841/2	a.	54	36
	b.	54	35
14247/8	a.	69	32
	b.	68	34
14249/50	a.	69	32
	b.	62	33
14605/6	a.	60	31
	b.	54	28
	c.	58	24
Average		55.6	29.0

B. THE AREA IN MM^2 OF 6 DAY OLD FIBROBLAST COLONIES (SISTER HALVES) GROWING IN THE PRESENCE OF 25% EXTRACT FROM ACETONE DESICCATED HEARTS (A), AND OF THE ACETONE PRECIPITATE OF EXTRACT FROM ACETONE DESICCATED HEARTS (B).

No. of cultures		A.	B.
13647/8	a.	34	31
	b.	34	34
13757/8	a.	48	31
	b.	36	24
13843/4	a.	42	24
	b.	45	34
14243/4	a.	56	34
	b.	48	38
14607/8	a.	42	28
	b.	42	27
	c.	40	26
14609/10	a.	48	24
	b.	44	26
	c.	47	25
Average		43.2	29.0

2. *The completely undenatured acetone precipitate has the same growth-promoting activity as the partially denatured precipitate.*

Table 2, summarizing the results of a comparison of the activities of an entirely undenatured acetone precipitate of adult chicken heart extract (prepared by acetone precipitation below —10° C.) and of

TABLE 1 (*continued*)

C. The Area in mm^2 of 6 Day Old Fibroblast Colonies (Sister Halves) Growing in the Presence of Tyrode's Solution (A), and of the Acetone Precipitate of Extract from Acetone Desiccated Hearts (B)

No. of cultures		A.	B.
13641/2	a.	12	25
	b.	13	25
15352/3	a.	8	32
	b.	8	38
15354/5	a.	12	38
	b.	11	39
15356/7	a.	10	50
	b.	10	31
15358/9	a.	8	35
	b.	10	44
	c.	9	42
Average		10.1	36.3

TABLE 2

The Area in mm^2 of 6 Day Old Fibroblast Colonies (Sister Halves) Growing in the Presence of a Partially Denatured Acetone Precipitate (A), and of an Undenatured Acetone Precipitate (B) of Extract from Acetone Desiccated Hearts

No. of cultures		A.	B.
14313/4	a.	48	32
	b.	40	33
	c.	28	23
14315/6	a.	34	27
	b.	25	20
	c.	30	26
14317/8	a.	35	31
	b.	26	24
	c.	27	20
14601/2	a.	33	31
	b.	32	31
	c.	28	33
14603/4	a.	25	30
	b.	28	32
	c.	33	33
Average		31.4	28.4

partially denatured precipitate (obtained at 0 to 5° C.), demonstrated that the undenatured precipitate is neither more nor less active than the partially denatured precipitate.

3. *Fractional precipitation of heart extract with acetone.*

Table 3 compares the growth-promoting effects of the precipitates obtained from heart extract by varying the concentration of acetone, with that of Tyrode's solution. It is clear that the most active precipi-

TABLE 3

THE AREA IN mm^2 OF 6 DAY OLD FIBROBLAST COLONIES (SISTER HALVES) GROWING IN THE PRESENCE OF TYRODE'S SOLUTION (A), AND OF THE PRECIPITATES OBTAINED WITH INCREASING CONCENTRATIONS OF ACETONE FROM EXTRACTS OF ACETONE DESICCATED HEARTS (B)

% of acetone	% of total material precipitated	No. of cultures		A.	B.
		13255/6	a.	18	26
			b.	24	23
9	2	13257/8	a.	10	12
			b.	16	17
		13261/2	a.	22	21
			b.	24	15
		Average		19.0	19.0
		13323/4	a.	12	19
			b.	13	20
23	11	13325/6	a.	10	16
			b.	10	23
		13297/8	a.	19	12
			b.	24	32
		Average		14.6	20.3
		13289/90	a.	14	38
			b.	11	31
33	36	13291/2	a.	9	61
			b.	10	61
		13293/4	a.	23	38
			b.	24	44
		Average		15.1	43.3
		13311/2	a.	10	16
			b.	13	21
41	24	13307/3	a.	11	26
			b.	14	31
		13309/10	a.	9	20
			b.	20	34
		Average		14.1	24.7
		13301/2	a.	16	26
			b.	14	28
47	17	13303/4	a.	14	15
		13305/6	a.	23	18
			b.	26	22
		Average		18.6	21.8
		13315/6	a.	15	18
			b.	20	27
60	8	13317/8	a.	11	12
			b.	19	21
		13319/20	a.	30	23
		Average		19.0	20.2

TABLE 3 (*continued*)

% of acetone	% of total material precipitated	No. of cultures		A	B.
		13333/4	a.	14	14
			b.	18	20
75	2	1333/5	a.	19	20
			b.	17	15
		Average		17.0	17.2

tates are those obtained at concentrations of 33 and 41% acetone. However, this does not imply any sharp separation of the active material by fractional precipitation with acetone under these conditions, since the largest amount of the total acetone-precipitable material is obtained within the same range of concentrations.

4. *The acetone soluble fraction of adult chicken heart extract has no growth promoting activity, when tested alone.*

Table 4 compares the effect of the acetone soluble fraction of heart

TABLE 4

THE AREA IN MM^2 OF 6 DAY OLD FIBROBLAST COLONIES (SISTER HALVES) GROWING IN THE PRESENCE OF TYRODE'S SOLUTION (A), AND OF THE ACETONE SOLUBLE FRACTION OF EXTRACT FROM ACETONE DESICCATED HEARTS (B)

No. of cultures		A	B.
13857/8	a.	18	16
	b.	15	18
13861/2	a.	18	22
	b.	13	18
13875/6	a.	19	24
	b.	15	16
13877/8	a.	14	22
	b.	19	14
13879/10	a.	19	16
	b.	16	19
13381/2	a.	18	24
	b.	18	18
13383/4	a.	20	16
	b.	19	15
13387/8	a.	18	16
	b.	20	28
14295/6	a.	13	24
	b.	16	23
	c.	21	23
14297/8	a.	18	23
	b.	19	25
	c.	18	20
Average		17.4	19.5

extract with that of Tyrode's solution. It is clear that the acetone soluble fraction, alone, possesses either very slight or no growth promoting activity in vitro.

5. *A mixture of acetone precipitate and the acetone soluble fraction is as active as the original heart extract.*

The acetone precipitate may be considered to contain the same concentration of acetone precipitable substances as the original extract, while the acetone soluble fraction may be considered to contain the same concentration of acetone soluble substances as the original extract. Thus, a mixture of 1 volume of acetone precipitate with 1 volume of acetone soluble fraction corresponds to a 50% dilution of the original heart extract as far as the concentration of the acetone precipitable and acetone soluble substances contained in it are concerned. Table 5, comparing the activity of such a mixture with a 50% dilution of the acetone precipitate, alone, and with that of a 50% dilution of the original heart extract, shows that: (a) the mixture is more active than the acetone precipitate alone (Table 5a), and (b) that it is as active as the original extract (Table 5b). It is thus clear that the addition of the inactive acetone soluble fraction to the partially active acetone precipitate, which possesses less than 25% of the original activity, results in a complete reconstitution of the original activity of the heart extract.

The partial recovery of activity in the acetone precipitate is obviously not due to partial protein denaturation caused by the precipitation, since (a) an undenatured precipitate is no more active than a partially denatured one (see 2 above) and (b) the full activity of the original heart extract is reconstituted by mixing the acetone precipitate with the acetone soluble fraction in the concentrations in which these fractions were present in the original heart extract.

Discussion

These results are strikingly parallel to those previously obtained in this laboratory by the dialysis of heart extract, the fully dialyzed heart extract having approximately the same range of activity as the acetone precipitate described above, and this activity being reconstituted to its original level by the addition of the inactive or only very slightly active dialysate, just as the activity of the acetone precipitate was raised to that of the original heart extract by the addition of the inactive acetone soluble fraction.

TABLE 5

A. The Area in mm^2 of 6 Day Old Fibroblast Colonies (Sister Halves) Growing in the Presence of the Acetone Precipitate of Extract from Acetone Desiccated Hearts (A), and of a Mixture of the Acetone Precipitate with the Acetone Soluble Fraction of Extract from Acetone Desiccated Hearts (B)

No. of cultures		A.	B.
13775/6	a.	36	52
	b.	44	44
13789/90	a.	36	45
	b.	36	39
13791/2	a.	32	50
	b.	30	48
13793/4	a.	22	50
	b.	28	78
13849/50	a.	36	45
	b.	36	48
13851/2	b.	33	50
13869/70	a.	28	50
	b.	28	56
13873/4	a.	40	54
	b.	43	56
14299/300	a.	38	72
	b.	36	54
	c.	40	46
14301/2	a.	24	62
14303/4	a.	34	45
	b.	26	48
	c.	43	68
Average		34.0	52.7

B. The Area in mm^2 of 6 Day Old Fibroblast Colonies (Sister Halves) Growing in the Presence of 50% Extract from Acetone Desiccated Hearts (A), and of a Mixture of the Acetone Precipitate with the Acetone Soluble Fraction of Extract from Acetone Desiccated Hearts (B)

No. of cultures		A.	B.
13761/2	a.	57	54
	b.	56	42
13763/4	a.	67	57
	b.	68	68
13765/6	a.	44	58
	b.	58	32
13845/6	a.	38	39
	b.	50	44
13847/8	b.	61	54
15360/1	a.	57	43
	b.	45	60
15364/5	a.	41	70
	b.	48	62
15366/7	a.	48	82
	b.	66	76
15368/9	a.	72	66
	b.	58	67
Average		55.0	57.3

The experiment described above, performed with extracts from acetone-desiccated adult chicken hearts, confirm and extend the observations of a number of authors, who have repeatedly separated protein and non-protein or micro- and macromolecular fractions from embryonic extracts, using a wide variety of methods. Thus, excluding those authors who have used dialysis as the method of separation and to whom reference was made in a previous paper (Margoliash, Tenenbaum and Doljanski, 1948), we find that:

Baker and Carrel (1926) precipitated the proteins of embryonic chick juice by bubbling CO_2 through a diluted extract or by the addition of acetic acid, ethyl-alcohol, methyl alcohol, isopropyl alcohol or acetone. With some precipitants the results were better than with others, but in every case the precipitate was less active than the original embryonic extract. The micromolecular portion of the extract separated by ultra-filtration showed no activity whatsoever.

Fischer (1930) confirmed the work of Baker and Carrel, and obtained the best results by alcohol precipitation, followed by washing of the precipitate with ether. Moreover, fractional precipitation with acetone yielded the most active portion with one-half volume of acetone (Fischer 1941). None of the fractions was as active as the original extract. The micromolecular components of the extract were also inactive. More recent work by the same author (Fischer and Astrup, 1943) led to the isolation of a nucleoprotein termed "embryonin" as the active component of the macromolecular component of beef embryo extract, and the replacement of the micromolecular fraction by a mixture containing inorganic salts, glucose, fructose diphosphate, 9 amino acids, cystine, glutathione, and glutamine (Fischer, Astrup, Ehrensvaerd and Oehlenschlager, 1948) or by the accessory growth factors obtained from a variety of natural sources, such as blood, serum, kidney, heart, yeast and barley malt (Astrup, Ehrensvaerd, Fischer and Oehlenschlager, 1947).

Tennant *et al.* (1941) isolated a macromolecular material from chick embryos by high speed centrifugation and this fraction was found to promote the growth of cultures of embryonic mouse heart. Since their material was not very active and was completely heat-stable, it is probably not identical with that separated by the precipitation methods of Baker and Carrel (1926) and Fischer (1930).

Doljanski and Werner (1945), using saline extracts of fresh adult chicken hearts, showed that alcohol precipitates displayed consider-

able growth-promoting power, and that this activity was not affected by ether, petroleum-ether or acetone extraction.

Davidson and Waymouth (1945) found that the alcohol precipitates obtained from embryonic extracts are more active than similar preparations from adult sheep heart in promoting the growth of fibroblasts, as measured by the increase in the nucleoprotein phosphorus, during the growth of fresh explants from 10 day chick embryo hearts.

The results presented in this paper show that:

1. The precipitate obtained by the addition of acetone to an extract of acetone-desiccated adult chicken heart contains somewhat less than 25% of the growth-promoting activity of the original extract. This apparent loss of activity is not associated with the protein denaturation that occurs during precipitation.

2. The fraction of heart extract that is soluble in acetone has no growth-promoting activity.

3. The acetone soluble fraction and the acetone precipitate when mixed possess the full growth-promoting activity of the original heart extract.

The acetone insoluble and acetone soluble fractions thus seem to correspond respectively to the non-dialysable and dialysable fractions of heart extract (Margoliash, Tenenbaum and Doljanski, 1948).

The very preliminary nature of both these experiments obviously precludes any speculation as to the nature of the substances responsible for these effects. However, the results obtained by acetone precipitation do lend further support to the previously expressed view (Margoliash, Tenenbaum and Doljanski, 1948) that the in vitro cell growth-promoting effect of adult tissue extracts depends on at least two factors or two series of factors acting together to produce the total effect.

Summary

The effect of acetone fractionation on the growth-promoting activity of adult tissue extracts was studied on an extract of acetone-desiccated adult chicken hearts. It was found that:

1. The acetone precipitate contains somewhat less than 25% of the original activity of the extract. This partial recovery of the activity in the precipitate is not due to the protein denaturation that occurs during the manipulation.

2. The acetone soluble fraction is inactive when tested alone.

3. Combining the acetone precipitate with the acetone soluble fraction results in complete recovery of the original activity.

4. Fractional precipitation with acetone causes no significant concentration of the activity of the acetone precipitate.

References

1. Astrup, T., Ehrensvaerd, G., Fischer, A., & Oehlenschlager, V. 1947. Protein metabolism of tissue cells in vitro. 6. The accessory growth substances. *Acta Physiol. Scand.*, **14**, 195-207.
2. Baker, L. E., & Carrel, A. 1926. Action on fibroblasts of the protein fraction of embryonic tissue extracts. *J. Exper. Med.*, **44**, 387-395.
3. ———. 1926. Effect of the amino acids and dialyzable constituents of embryonic tissue juice on the growth of fibroblasts. *J. Exper. Med.*, **44**, 397-407.
4. Davidson, J. N., & Waymouth, C. 1945. Nucleoprotein content of fibroblasts growing in vitro; effect of tissue extracts. *Biochem. J.*, **39**, 188-199.
5. Doljanski, L., & Doljanski, F. 1944. Studies on the growth promoting factor of adult tissue extracts. I. The effect of heat. II. The effect of dilution. *Growth*, **8**, 99-106.
6. Doljanski, L., & Werner, H. 1945. Studies on the growth-promoting factor of adult tissue extracts. III. Precipitation with alcohol. IV. Action of lipid solvents. *Growth*, **9**, 229-234.
7. Fischer, A. 1930. Gewebezuechtung. Muenchen: Mueller and Steinecke, p. 22.
8. ———. 1941. The nature of the growth-promoting substances with embryonic tissue juice. *Acta Physiol. Scand.*, **3**, 54-70.
9. Fischer, A., & Astrup, T. 1943. Untersuchungen ueber die chemischen Eigenschaften des tierischen Wuchsstoffes aus Embryonen. *Pflügers Arch. f. d. ges. Physiol.*, **247**, 34-52.
10. Fischer, A., Astrup, T., Ehrensvaerd, G., & Oehlenschlager, V. 1948. Growth of animal tissue cells in artificial media. *Proc. Soc. Exper. Biol. & Med.*, **67**, 40-46.
11. Margoliash, E. 1947. Acetone-desiccated adult tissues as a source of cell growth-promoting extracts. *Science*, **105**, 369.
12. Margoliash, E., Tenenbaum, E., & Doljanski, L. 1948. Studies on the growth-promoting factor of adult tissue extracts. V. The effect of dialysis. *Growth*, **12**, 1-13.
13. Tennant, R., Liebow, A. A., & Stern, K. G. 1941. Effect of Macromolecular Material from Chick Embryos on Growth Rate of Mouse Heart Fibroblast Cultures. *Proc. Soc. Exper. Biol. & Med.*, **46**, 18-21.

Growth, 1950, **14**, 31-48.

THE EFFECT OF CORAMINE ON MITOTIC ACTIVITY AND GROWTH IN THE LIVER OF THE MOUSE[1]

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INTRODUCTION

That coramine included as 1% of the diet of young rats causes a marked increase in size of the liver was reported by Coulson and Brazda (1947). In a more detailed study (Brazda and Coulson, 1948) they showed that the increase occurs rapidly, within 5 days. It is not due merely to increase in water, fat or glycogen content, but involves both solids and water in about the same proportion as in normal liver. A prominent feature of the liver parenchyma is a large number of mitotic figures. A true growth of the liver tissue therefore occurs. They at first suggested the possibility that this is a regenerative process following an injury to the liver cells by the coramine. Since no injury was observed after 28 days this seemed doubtful, and an alternative suggestion, that the rapidity and degree of enlargement superficially resembles the effect produced in organs by their respective trophic hormones, was tentatively proposed.

The experiments here reported were undertaken in connection with an extensive study of the nuclear phenomena of the liver of the mouse. We have repeated and confirmed the work of Brazda and Coulson on the young, rapidly growing animal. We have also found an initiation of mitotic activity in the livers of adult mice when coramine is included in the diet. Mice that have been accustomed to the coramine-containing diet have lived on it for many months, grown to adult size, and have mated and produced and raised litters successfully. There

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have been no general indications of deleterious effects of the diet that cannot be attributed to a slightly lowered food intake that seems to be due to the fact that the mice find the diet distasteful. Cytological studies have shown that the enlargement of the livers and increased mitotic activity are associated with a mild hydropic vacuolization of the cytoplasm of the liver cells, particularly in the central part of the lobule.

Material and Methods

The mice were from inbred strains of albino mice that are being developed in our laboratory. The colony is housed in a room with temperature controlled at 75-78 degrees Fahrenheit and is fed on Purina Laboratory Chow.

Coramine has been administered by adding it to the diet. Other methods of administration have been tried—by esophageal tube, by subcutaneous injection, and in drinking water—but have been unsatisfactory. The following semisynthetic diet has been used routinely: vitamin-test casein (G.B.I.) 30, cornstarch (Argo) 28, dried brewer's yeast (G.B.I.) 8, salt mixture (USP XII #2) 7, Crisco 20, corn oil (Mazola) 4, cod liver oil (Squibb) 2. When a low fat diet was used, the Crisco was replaced by cornstarch. As a rule, coramine prepared for oral administration rather than for injection has been used. Because the coramine acts as a wetting agent, its incorporation into the diet has presented some difficulty. We have tried several methods and found the following the most satisfactory: the coramine, a 25% aqueous solution, is diluted with alcohol and evaporated onto G.B.I. vitamin-test casein. In its preparation, this casein is alcohol-extracted, and with it a fine powder can be obtained, whereas with edible casein there is a troublesome formation of gummy lumps.

In all types of experiments there has been some variability which we have come to attribute to variability in food intake. It is difficult to tell whether this is due to toxicity of the coramine or merely to an unpleasant taste.

The commercial preparation of coramine for oral administration contains lactic acid to counteract the unpleasant taste. Attempts to improve the flavor of the diets by substituting sugar for cornstarch, or protein hydrolysate or dried liver for part of the casein, have not met with notable success.

For microscopic study the livers have been routinely fixed in Bouin's

fluid, sectioned in paraffin at 5 μ and stained in Delafield's hematoxylin and eosin. For the study of fat they have been fixed in Baker's formol-calcium, sectioned on the freezing microtome at 5-15 μ, and stained with Sudan black or Sudan IV. For the study of glycogen they have been fixed in Rossman's picric acid-alcohol-formalin mixture, sectioned in paraffin at 5 μ, and stained by the Bauer-Feulgen or the McMannus (periodic acid-Schiff) technic. For the study of cytoplasmic basophilia they have been fixed in Zenker's fluid, sectioned in paraffin at 5 μ and stained with a standard methylene blue solution with pH controlled at 8.1. In those experiments in which counts of mitotic figures were made, the mitotic activity was computed according to the method described by Wilson and Leduc (1947).

Experimental

The experiments here reported are presented in 4 groups: (1) those with young mice in which mitotic activity is still normally present in the liver, (2) those with adult mice in which mitotic activity in the liver has normally ceased, (3) those with young mice raised to maturity on the diet containing coramine, and (4) those in which reproduction and lactation have been studied.

Experiments with young mice. A group of 8 experiments was conducted, 5 using 18- to 20-day-old mice, and 3 using 28- to 33-day-old mice. One to 3 litters of 7 or 8 mice were used in each experiment. One or more animals were sacrificed as untreated controls, and the remainder were sacrificed at intervals following the addition of coramine to the diet. Liver sizes and mitotic activities of all the animals in 5 experiments are presented in Table 1. Because of the great individual variability, the results have not been treated statistically.

The weight of the liver of an untreated young mouse is usually 5.5 to 6.0% of the total body weight of the animal, but it may vary from 4.5 to 7.5%. In the 90 animals of the 8 experiments, killed 2 to 50 days after addition of 1% coramine to the diet, liver weights varied from 5.5 to 9.6%, with a majority between 7.0 and 8.5% of total body weight. Increased liver weight/body weight ratio over that of untreated littermates occurred as early as 3 days following coramine administration, and persisted through at least 50 days.

A marked increase in mitotic activity over that in control littermates has been found as early as two days and as long as 38 days

TABLE 1

MITOTIC ACTIVITY AND LIVER WEIGHT/BODY WEIGHT RATIO IN YOUNG MICE RECEIVING 1% CORAMINE DIET

Under each experiment, the first column gives the liver weight/body weight ratio, and the second the mitotic activity of each mouse.

Days on diet	Exp. 231 26 days of age		Exp. 260 26 & 29 days of age		Exp. 426 18 days of age		Exp. 449 19 days of age		Exp. 511 32 & 33 days of age	
1	—	0.55	5.7%	0						
2	—	7.34	5.6%	0.67	5.5%	1.59				
3	—	0.3	8.2%	2.2	6.2%	2.67				
4	—	0.66	7.1%	0.3	5.6%	1.6	8.3%	4.84	9.0%	2.36
									7.5%	0.29
5	—	1.76	7.4%	0.6	5.4%	2.56	8.1%	0.75		
6	—	0.99	7.1%	0.5	4.8%	4.86				
7	—	6.73	7.9%	4.1	6.4%	1.9	8.7%	1.15		
8	—	15.14	8.6%	1.9	6.0%	2.69				
9							9.6%	5.35	8.0%	2.6
									8.7%	2.9
									7.3%	2.6
									8.2%	2.5
									7.8%	1.9
10			7.1%	2.7						
11							9.2%	2.17		
12	—	2.83	8.2%	4.3						
14									7.9%	2.2
									8.2%	0.6
									6.5%	0.3
									7.7%	1.6
									8.0%	1.2
									8.1%	2.5
15	9.0%	0	8.2%	3.3			9.5%	0.59		
20	7.7%	6.39	7.4%	1.4						
25	6.1%	0								
28	7.3%	0.97								
30	6.5%	2.46								
Controls										
0	—	0.58	—	1.88	4.8%	0.92			6.8%	0.24
4							7.7%	0.12		
8	—	1.4								
15							7.1%	0		
17	5.8%	1.5								
31	5.5%	0.12								

after the addition of coramine to the diet. Mitotic activities of as much as 10 times that of control animals have been found. The appearance of mitosis in coramine-treated animals is highly unpredictable. There is no gradual increase to a peak of activity and subsequent decline during a given period of time. There is no correlation between size of the liver and its mitotic activity; indeed, the largest livers may contain very few figures, and the highest levels of activity may be

found in livers that are not significantly larger than those of control animals. In a series of animals killed at daily intervals during the coramine regime, high levels of mitotic activity may alternate with low levels. Furthermore, neither enlargement of the liver nor increased mitotic activity is dependent on increased body weight.

Experiments with adult mice. Four experiments were conducted in which the animals were 130, 160, 177 and 250 days of age. No mitotic figures were found in the livers of untreated littermates. The mitotic activities and liver sizes in these experiments are presented in Table 2.

TABLE 2

MITOTIC ACTIVITY AND LIVER WEIGHT/BODY WEIGHT RATIO IN ADULT MICE RECEIVING 1% CORAMINE DIET

	Mitotic Activity			Liver Weight/Body Weight Ratio		
Days on diet	Exp. 232[1] 160 days of age	Exp. 259 250 days of age	Exp. 304 177 days of age	Exp. 259 250 days of age	Exp. 304 177 days of age	Exp. 483[2] 130 days of age
Control	0	0	0	5.5%	5.0%	4.9%
1	0	—	0	—	4.9%	5.0%
2	0	10.75	0	6.6%	5.8%	4.9%
3	0	—	0.34	—	6.1%	5.3%
4	0	0.2	0	4.7%	6.2%	4.8%
5	0.1	0	0.17	5.3%	4.9%	5.5%
6	4.5	0.2	0.33	4.9%	4.6%	4.3%
7	0.6	0.1	1.62	5.5%	6.4%	6.0%
8	0.4	0	0	6.3%	5.1%	6.2%
10		0.2	1.47	5.8%	5.6%	5.7%
12		0	0.39	5.9%	5.8%	5.1%
14		0	0.45	6.7%	6.7%	6.3%

[1]Liver weights for Exp. 232 are not available.
[2]Mitotic figures present only on 10th, 12th and 14th days.

The results in general have been positive in that, in many cases, mitosis has been induced and, in a few cases, a spectacular amount has occurred. The enlargement of the liver which is so striking in young, growing animals has not generally been found in the adult animals. In only a few instances have the livers seemed to be at all large. As in the young mice, the appearance of the mitotic figures is not constant and cannot be predicted on the basis of the amount of food or coramine intake and the change in body or liver weight.

In a fifth series of 163-day-old mice, which were killed 2 to 9 days after the addition of coramine to the diet, mitosis was found in only 2 animals, and only a very small number of figures in these. In a

heterogeneous group of very old mice, considerable mitotic activity was induced by the addition of 1% coramine to the diet. Two animals, 447 and 571 days of age, killed on the fifth and seventh days of coramine ingestion, had enlarged livers, 8.1% and 8.5% of their total body weight. There were no mitotic figures in the liver of the former and a very large number in the latter.

Experiments with young mice raised to maturity on coramine-containing diets. Two groups of mice, 12 that were 18 days old and 16 that were 21 days old, were weaned onto semi-synthetic diets containing 1% coramine and raised to maturity before sacrifice. Both low and high fat diets were used in the first group, but we found no significant differences that could be attributed to the fat content of the diet. Pairs of animals in the first group were sacrificed after 22, 53, 69 and 84 days on the diets and, up to the time of writing, individual mice in the second group had been sacrificed after 28, 107, 140, 176 and 210 days on the diet.

The growth of the animals raised on the 1% coramine diet was essentially normal. In the first group of mice there was an initial failure of growth during the first 5 days in those receiving coramine, while their littermates receiving the diets without coramine grew very rapidly. Subsequently, however, the experimental animals increased in weight at about the same rate as the control animals. The weight gains leveled off to a more gradual rise after 3 to 4 weeks, and at this time the body weights of the animals receiving coramine approached, and in some cases exceeded, those of controls. In the second group of mice there was no initial failure of growth in the animals that were fed the 1% coramine diet (Fig. 1). The growth of the experimental animals paralleled that of the control animals from the time they were given the diets through at least 210 days.

The increased liver weight/body weight ratio observed in young, growing mice persists when these animals are maintained on the 1% coramine diets for long periods of time. All the animals in these two experiments that were sacrificed at intervals from 22 to 210 days had enlarged livers (Table 3). They differ in this respect from the adult mice maintained on coramine for short periods of time, in which no liver enlargement was found. Mitotic activity in the livers of all animals, except those killed 22 days after the addition of coramine to the diet, was no greater than is found in the livers of untreated mice

of the same age; that is, not more than one or two figures were present in a standard liver section. Thus, from these experiments and from those above with young mice, it appears that the stimulatory effect of coramine on liver mitosis does not last more than 4 or 5 weeks.

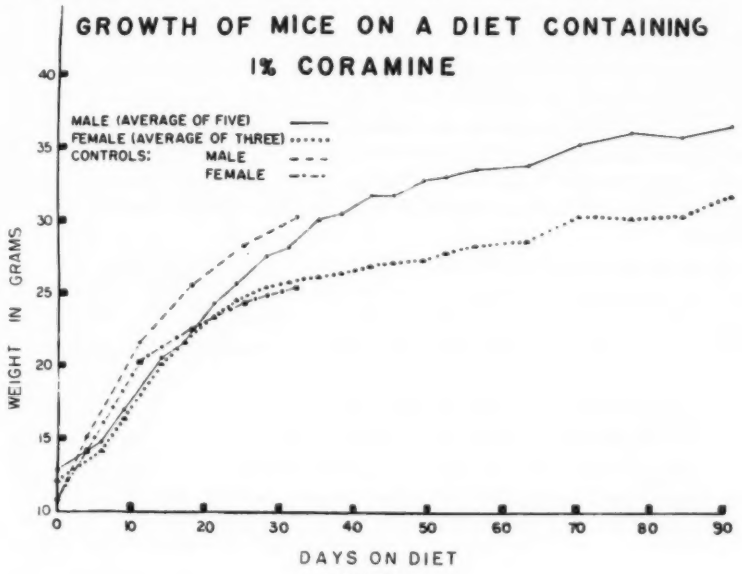

FIGURE 1

TABLE 3

LIVER WEIGHT/BODY WEIGHT RATIO IN MICE RAISED ON 1% CORAMINE DIETS

A		B		C		D	
Days on diet	Liver weights	Days on diet	Liver weights	Days on diet	Liver weights	Days on diet	Liver weights
22	5.8%	22	8.5%	28	7.8%	23	6.2%
22	5.9%	22	8.6%	107	7.2%	29	8.0%
53	6.1%	53	8.9%	140	6.3%	136	7.6%
53	5.6%	53	8.2%	176	9.2%	168	6.9%
		69	10.3%	210	8.0%	168	9.2%
		69	6.0%				
		84	6.4%				

A. Animals weaned onto control diet without coramine at 18 days of age.
B. Animals weaned onto 1% coramine diet at 18 days of age.
C. Animals weaned onto 1% coramine diet at 21 days of age.
D. Offspring of animals raised on 1% coramine diet.

Experiments on reproduction and lactation. Two pairs of mice that had been raised on 1% coramine diets were mated and 6 litters of 9 to 11 offspring were born. One of the females had 4 litters which were born after she had been on the coramine regime for 44, 76, 128 and 199 days. The young appeared normal in every respect, but few survived to weaning because of an apparent inability of the mothers to nurse them. Whether or not this is due to the inadequacy of the semisynthetic diet has not been established. In 4 of the litters, one to 4 of the offspring have survived, however, although none grew as rapidly as the offspring of untreated females (Fig. 2). These young have been weaned onto the coramine diets. Some have survived as long as 168 days before sacrifice, and one pair has, in turn, produced a litter.

Five offspring of coramine-fed animals that were weaned onto the 1% coramine diet have been killed at the ages of 23, 29, 136 and 168 days. All had enlarged livers (Table 3). Mitotic figures were abundant only in the animals that were killed when 23 and 29 days old.

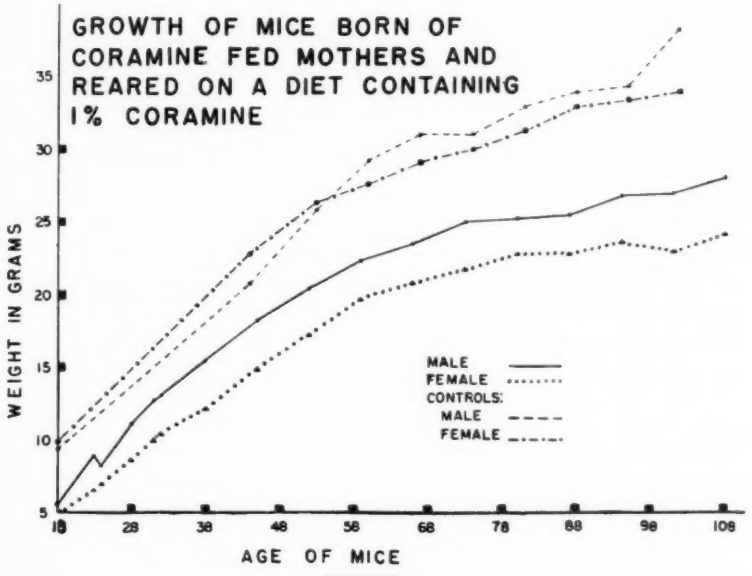

FIGURE 2

Cytological observations. There is no necrosis of the liver in either the short term or long term experiments. A constant feature, however, in the livers of all mice receiving 1% coramine in the diet, is the appearance of a foam-like, alveolar vacuolization of the cytoplasm. We have interpreted this as a hydropic vacuolization. The vacuoles are at first small and spherical (Fig. 4), but become progressively larger and irregular in form. They are separated by very thin partitions of cytoplasm. The hydropic cells may or may not contain glycogen or fat or both. When present, the glycogen appears as discrete fine granules (Fig. 7), and the fat as small droplets. Both are dispersed in the cytoplasmic partitions between the vacuoles. The occurrence of both in the livers of coramine-fed animals is in keeping with the report of Brazda and Coulson (1948). Associated with this cytoplasmic vacuolization is a distinctive clumping of the basophilic material of the cytoplasm. The aggregates of this material represent cytoplasmic ribonucleoprotein, which after acid fixation appear to be made up of abundant slender filaments arranged in more or less parallel rows (Fig. 8). The basophilic material frequently seems more abundant in the hydropic cells than in the corresponding cells of control livers, and, in contrast with the vacuolated areas of the cytoplasm, gives the cell a mottled appearance (Figs. 3 and 6). Hydropic cells frequently exhibit an enlargement and vacuolization of the nucleoli and, occasionally, a distention of the nucleus with a decrease in the chromaticity of the nuclear sap and a distention of the entire cell. In many of the animals, in the region of the lobule containing hydropic cells, the bile canaliculi are distended, sometimes strikingly so (Fig. 6).

Cells showing these hydropic changes have been found as early as two days after the initiation of coramine feeding. There are individual differences in the distribution of hydropic vacuolization in the lobule, but it can be found in nearly all animals receiving coramine. It is at first limited to the cells in the central region of the lobule, but after a long period of treatment it may extend well into the peripheral area.

When mitosis is abundant, a large number of the figures are usually in the hydropic cells (Figs. 3, 4 and 5).

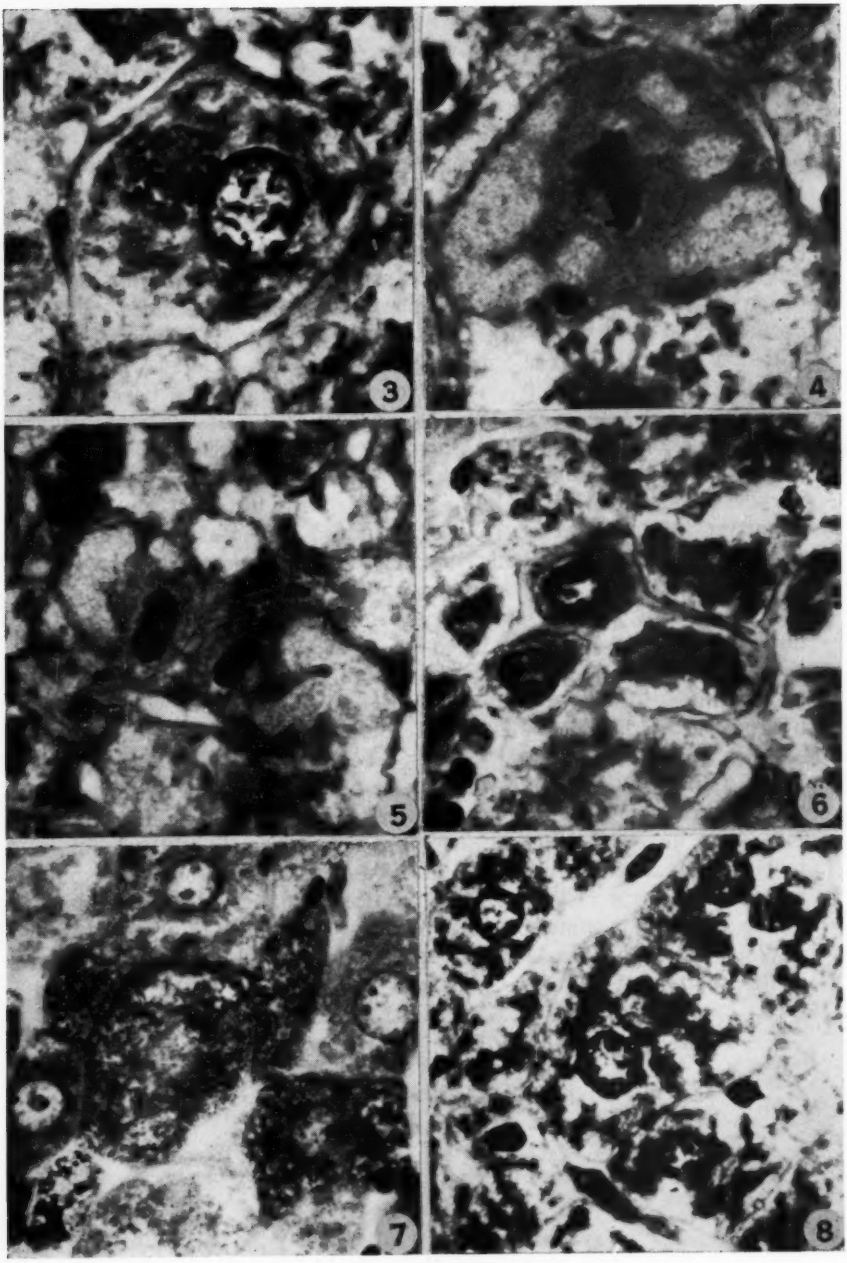
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PLATE I

Explanation of Figures

Figures 3-6 are from mouse liver fixed in Bouin's fluid, sectioned in paraffin at 5μ, and stained in Delafield's hematoxylin and eosin. Figure 7 is from a liver fixed in Rossman's picro-alcohol-formalin and stained for glycogen by the Bauer-Feulgen technic. Figure 8 is from a liver fixed in Zenker's fluid and stained in M/16,000 methylene blue at pH 8.1.

All photomicrographs were taken at a uniform magnification with a 2 mm. apochromatic oil immersion objective and a 7.5 × compensating ocular.

Fig. 3. A binucleate cell in prophase. The cytoplasm contains fine alveolar vacuoles and irregular, clumped basophilic masses. From a 42-day-old mouse that had been 9 days on a diet containing 1% coramine.

Fig. 4. A metaphase figure in a cell containing hydropic vacuoles. The basophilic component of the cytoplasm is clumped about the spindle. From a 42-day-old mouse that had been 9 days on a diet containing 1% coramine.

Fig. 5. A late telophase with completed cytoplasmic furrow in hydropic cells. A large bile canaliculus appears in cross-section between the daughter cells. From the same animal as Fig. 4.

Fig. 6. Distended bile canaliculi in a hydropic zone of the liver lobule. From a 36-day-old mouse that had been 6 days on a diet containing 2% coramine.

Fig. 7. Fine, discrete granules of glycogen in the strands of cytoplasm around the hydropic vacuoles. Note the enlarged and vacuolated nucleoli. From a 46-day-old mouse that had been 28 days on the diet containing 1% coramine.

Fig. 8. Basophilic fibrils aggregated in irregular clumps in the cytoplasm of hydropic liver cells. From a 168-day-old mouse born and raised on a diet containing 1% coramine.

Discussion

Our primary interest has been in the effect of coramine on mitosis in the liver. Mitotic activity ceases in the liver of the mouse from 4 to 5 weeks after birth, and is rarely found in the liver of the adult. The liver cells are, however, still capable of mitotic activity. During repair after partial hepatectomy or after injury due to hepatotoxic agents such as phosphorus or carbon tetrachloride, mitotic activity is very high.

There are few methods of stimulating mitotic activity in the liver that do not involve destruction of cells. We have studied and reported on three: the intraperitoneal injection of pulped homologous liver and related substances (Wilson and Leduc, 1947), the injection of thyroxin (Wilson and Leduc, 1948) and refeeding of mice after complete or partial inanition (Leduc, 1949). When coramine is added to the diet there may be an increase of mitotic activity in young animals or an initiation of mitotic activity in older ones. There is no destruction of cells. In general, there is associated with the increased mitotic activity an increase in liver weight/body weight ratio.

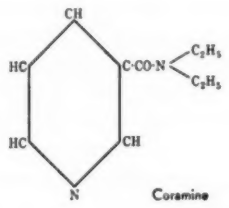
There is, however, considerable variation in the effect in our experiments. Neither the amount of mitotic stimulation nor the enlargement of the liver is constant. The livers of most of the young animals are enlarged, although some are not, whereas those of the adults are rarely significantly enlarged, although a few are. The variation is so great and so sporadic that the figures do not justify statistical analysis. Brazda and Coulson (1948) give their figures as averages of groups, but it is to be noted that in two different experiments they obtained strikingly different results in liver enlargement, which they tentatively attributed to the fact that the experiments were done at different times of the year. Although our experiments have been done at all seasons, they have been conducted in air conditioned rooms, so that seasonal variations should be minimized. The variation seems, nevertheless, to be greater in our experiments than in theirs.

We early became aware that there is considerable variation in the food intake of the mice as soon as they are placed on a coramine-containing diet. This takes place so suddenly that it could hardly be attributed to toxicity. We have attributed it to the taste of the coramine. It may well be that mice are more fastidious eaters than rats and that this explains why the effect on mice is more variable. Young

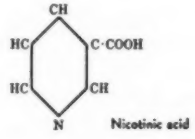
mice that are weaned onto the diet generally eat it more readily than do older animals that have become accustomed to other solid food. Some of these young mice have eaten the coramine-containing diet so well that they have grown and apparently thrived for months. One female that has borne 4 normal litters has eaten over half of her own weight in coramine, and is still thriving after 300 days on the diet. It is possible that if the mice ate the diet readily and uniformly, much of the variability of our experiments would disappear. But all of our attempts to improve the flavor of the diets by concealing the taste of the coramine have so far been unsuccessful.

The question of toxicity must, however, be considered, not only because loss of appetite is usually considered as an indication of toxicity when a drug is included in the diet, but also because under certain circumstances coramine is indeed toxic, and if its toxic action affected the liver, this might, as in the case of other hepatotoxic agents, explain the mitotic activity that follows its ingestion.

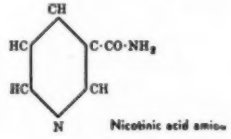
Coramine is chemically related to nicotinic acid and nicotinamide. The structural formulae are as follows:



Coramine



Nicotinic acid



Nicotinic acid amide

Brazda and Coulson (1946) have investigated the toxicity of these substances, and have shown that coramine, when administered by subcutaneous injection, is very toxic. We have found that a sufficiently large dose administered by injection or by esophageal tube is lethal to the mouse. When it is supplied in the diet, however, mice have eaten in a day several times the amount that would be sufficient to produce death in a single dose by stomach tube.

As far as the experiments here reported are concerned, the only effects that could be attributed to toxicity are decrease of food intake

with loss of weight or decrease in growth rate, and in some animals poor lactation with corresponding failure to raise litters satisfactorily. All of these effects could well be due to low food intake alone, and this could be due as well to the bitter taste of the coramine as to toxicity. We have never seen animals that are being fed coramine in the diet exhibit the symptoms that follow when it is injected. On the other hand, animals that become accustomed to the diet begin to eat more, and to grow better, and have successfully raised young even if not at an optimum rate. And young animals that are weaned onto the 1% coramine diet seem to be quite undisturbed by it. Ovulation and spermatogenesis are normal, and embryos develop normally in mothers on the diet. It cannot, therefore, be considered extremely toxic at the level we are feeding it. Furthermore, there is no evidence that its toxicity, when injected or fed alone by stomach tube, is directly upon the liver. It appears to act as a poison to the medullary respiratory center (Brazda and Coulson, 1946) rather than as an hepatotoxic agent.

In the histological study of the livers of animals receiving coramine, we have consistently found evidence of intracellular edema. The cytoplasm has a fine, foam-like alveolar structure with a clumping of the basophilic material which may give the cells a mottled appearance (Figs. 3 and 6). Cells of this type are considerably enlarged. The nuclei may also be enlarged, with nuclear sap lightly staining compared to normal nuclei. The nucleoli may be considerably distended, and are frequently vacuolated. These changes are completely reversible without destruction of cells. They seem to represent a mild form of the more striking intracellular edema of the "balloon cells" in the liver in chloroform and carbon tetrachloride poisoning, or the watery vacuolization of the liver described by Trowell (1946) after conditions of severe anoxia. Trowell found even the severe vacuolization he describes to be reversible. It would seem, then, that the hydropic changes we have found are well within the limits of physiological change, although it is difficult to tell when such a change could be described as "incipient injury".

A striking feature of the areas where these mottled cells appear is a distention of the bile capillaries (Fig. 6). Because the capillaries of the most swollen cells are usually the most distended, the distention would seem to indicate an increased flow of bile from these hydropic cells rather than an obstruction to the flow of bile.

The unusual distribution of the cytoplasmic basophilia is accentuated with increasing hydropic distention of the cells (Fig. 8). This material represents the cytoplasmic ribonucleoprotein. It is, in normal livers, found in the form of very fine filaments after acid fixation, and these may be scattered, as they usually are in the portal cells, or aggregated into clumps of a more or less indefinite configuration in the central cells (Deane, 1946). It tends to disappear in the early stages of starvation (Leduc, 1949), and very quickly after carbon tetrachloride administration (Rosin and Doljanski, 1946). It has been considered to be associated with enzyme activity, possibly with protein synthesis within the liver cells. The total amount seems to be increased in these hydropic cells, for the area in which they occur seems to be generally more basophilic than the rest of the liver under the low power of the microscope. The presence of this basophilic material in abundance would seem to argue against the idea that the coramine seriously injures the cells.

In many of the livers in which mitosis is abundant, it seems to be limited almost exclusively to the hydropic cells. The extensive hydropic vacuolization does not seem to interfere with the normal progress of mitosis. Normal figures, including telophases with complete separation of the daughter nuclei and cleavage of the cytoplasm, are abundant (Figs. 3, 4 and 5). Many abnormal figures have also been found, but they are no different from those we have described in animals subjected to other treatments or, indeed, in apparently normal animals (Wilson and Leduc, 1950). That the hydropic cells are capable of mitotic division argues further against the idea that they are seriously injured.

It would seem, then, that the hydropic cells are not injured structurally, nor are they seriously injured functionally. This conclusion is of importance in considering the mechanism by which coramine brings about the increase in mitotic activity and growth in the liver. If the hydropic cells are structurally damaged, the mitotic activity might be comparable to repair after injury. There is no injury to justify this view. If the hydropic cells are incapable of performing their normal functions, they might be considered to be, temporarily at least, as effectively removed as if they had been killed by an hepatotoxic agent, or actually removed by partial hepatectomy. The growth and mitosis might then be considered a compensatory hyperplasia of the remaining tissue. An explanation of this sort was given by Pfuhl

(1938) for mitotic activity in the livers of guinea pigs after injection of trypan blue.

If the distention of the bile capillaries indicates persistent flow of bile, this function at least must still be possible, even if abnormal. Cytochemical tests have shown glycogen in moderate abundance (Fig. 7), and, not infrequently some fat in these cells, which would seem to indicate a persisting function. There seems, then, to be no evidence of loss of function of these cells, and considerable presumptive evidence that most of their functions are maintained.

Furthermore, it is difficult to consider these cells injured and incapacitated, for they are the very ones that are most active mitotically. They resemble the cells of the intact parenchyma that participates in compensatory hyperplasia in livers damaged by hepatotoxic agents, for these cells also become both hydropic and mitotically active. It would seem then that coramine somehow induces in some of the cells of the liver an hydropic change resembling that in the intact parenchyma of an injured liver, and in both cases the hydropic change leads to mitotic activity. It is to be emphasized that such concepts as "compensatory hyperplasia", that have been used to account for the mitotic activity and growth in the intact parenchyma, are in reality teleological and explain nothing. Although the mitotic activity is associated with hydropic change, mitotic activity eventually subsides even if the coramine feeding is continued and the hydropic vacuolization persists. This suggests the possibility that coramine is not a general mitotic stimulant, but that in inducing the hydropic change, it releases mitotic processes which have been arrested in the liver cell, or prevents their arrest in the younger mice. Since in liver regeneration mitosis is sometimes similarly associated with hydropic vacuolization, it seems possible that this association may in general be of fundamental importance in the initiation or stimulation of mitosis in the liver.

Summary and Conclusions

The inclusion of 1% of coramine in the diet of young mice is followed by an increase in mitosis and an increase in liver weight/body weight ratio. In the diet of adult mice, it may initiate mitosis, but only rarely induces a significant increase in liver size.

The effect is highly variable, which is tentatively attributed to the fact that the mice eat the coramine-containing diet reluctantly.

Mice that have become accustomed to the diet have grown to maturity, mated, and raised young that have, in turn, grown to maturity on the diet.

Coramine is toxic when sufficiently large doses are injected or fed by esophageal tube. It is not, however, an hepatotoxic agent, but acts on the respiratory center of the brain. When fed at a level of 1% in the diet, the only symptoms are loss of weight or decreased growth rate and poor lactation. These could as well be attributed to poor food intake as to toxicity. The excellent growth of those animals that become accustomed to the food argues against toxicity.

The only cytological change of significance in the liver is an intracellular edema which may persist for months if the coramine regimen is continued, but which is completely reversible and which produces no cell destruction. The hydropic cells are distended with vacuoles, and the nuclei and nucleoli may be enlarged. The cytoplasmic basophilia may be increased and the bile capillaries distended. These facts indicate that the cells are not damaged and that they are still functional. The mitotic activity cannot therefore be attributed to compensatory hyperplasia.

It is in these hydropic cells that the mitotic figures are most frequently found. Since, in liver regeneration after injury by hepatotoxic agents or after partial hepatectomy, the mitotically active cells are frequently hydropically distended, it is suggested that this association may be of fundamental importance in the initiation or stimulation of mitosis in the liver.

References

Brazda, F. G., & Coulson, R. A. 1946. Toxicity of nicotinic acid and some of its derivatives. *Proc. Soc. Exp. Biol. and Med.*, **62**, 19-20.

———. 1948. The influence of coramine on the liver of the young rat. *Proc. Soc. Exp. Biol. and Med.*, **67**, 37-40.

Coulson, R. A., & Brazda, F. G. 1947. Effect of feeding pyridine derivatives to young rats on a high protein diet. *Proc. Soc. Exp. Biol. and Med.*, **65**, 1-5.

Deane, H. W. 1946. The basophilic bodies in hepatic cells. *Am. J. Anat.*, **78**, 227-244

Leduc, E. H. 1949. Mitotic activity in the liver of the mouse during inanition fol lowed by refeeding with different levels of protein. *Am. J. Anat.*, **84**, 397-430.

Pfuhl, W. 1938. Die mitotischen Teilungen der Leberzellen im Zusammenhang mit den allgemeinen Fragen über Mitose und Amitose. *Zeitschr. Anat. u. Entwicklungsgesch.*, **109**, 99-133.

Rosin, A., & Doljanski, L. 1946. Pyroninophilic structures of liver cells in carbon tetrachloride poisoning. *Proc. Soc. Exp. Biol. and Med.*, **62**, 62-64.

TROWELL, O. A. 1946. The experimental production of watery vacuolation of the liver. *J. Physiol.*, **105**, 268-297.

WILSON, J. W., & LEDUC, E. H. 1947. Mitotic rate in mouse liver following intraperitoneal injection of liver, kidney and egg yolk. *Anat. Rec.*, **97**, 471-494.

———. 1948. The effect of thyroxin on mitotic activity in the liver of the mouse (Abstract). *Anat. Rec.*, **100**, 724.

———. 1950. Abnormal mitosis in mouse liver. *Am. J. Anat.*, **86**, 51-74.

Growth, 1950, **14**, 49-80.

BODY SIZE AND ORGAN WEIGHT

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The organ weights of experimental animals have no significance as absolute values; they must first be related to body weight, or even to some chosen constant body weight. Two obvious considerations should determine the selection of the terms in which organ weight data are to be presented. One is that the reader should be provided with the means whereby he may, without too much labor, translate the data into terms comparable with the findings of other investigators who have used other methods of expression. The other, not infrequently the only one remembered, is that that method of expression should be chosen which is found to give the minimum variability for purposes of prediction.

The neglect of the first consideration has led to confusion and loss of painstaking effort. These difficulties are manifest in Kleiber's review of ten papers on metabolic rates in rats; the usual unit was surface area, but the way of calculating this was omitted by four authors, and by others consisted variously in taking the body weights to either the 2/3 or 3/5 power and multiplying by different factors like 7.42, 9.1, 10, or 12.44. Equivalent examples might be given for reports on the organ weights of rats. These are sometimes expressed in terms of body surface, derived in varying ways, and sometimes in terms of empirical formulae of varying degrees of complexity. The result is that any direct comparison of data is difficult, and when editorial pressure on authors to condense their tables leads to the omission of the absolute body weights and organ weights, comparison becomes impossible.

This conflict between general understandability and precision is a

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consequence of the current lack of agreement as to the general laws of the growth of the body and of its organs, and it can be overcome only by striving to attain the maximum of both, not by sacrificing either for the sake of the other, and certainly not by sinking back into the easily comprehensible but erroneous simplicity attained when organ weights are given as percentages of body weight. Such archaic modes of expression may be forgiven when one is dealing with organ weight data in new species where as yet there has been no possibility of any general survey. In the case of the rat, however, there exists a wealth of data on organ weights and body weights, so that it is possible to find modes of expression which have a high prediction value and yet are simple.

When the absolute weights of certain organs, as for instance liver, kidney, heart in both male and female, and the adrenal gland in the male, are plotted against body weights, the stream of points is curved, flattening out as body-weight increases. Such a parabolic plot can often be straightened out by a simple mathematical transformation, so as to yield a straight line. One of the commonest transforms is the log log relation. That is, we take the logarithm of organ weight and the logarithm of body weight, and plot one against the other; thus the curved stream is rectified to a straight line, which is much more convenient. The logarithms are then our working units and are used for the further calculations of the equation which defines that straight line.

The concise algebraic expression of the curved stream is a parabola $y = bx^k$, where x = the independent variable, namely the body-weight in grams, y = the dependent variable, namely the organ weight in milligrams, k = slope of the line, or growth rate constant, and b = the initial growth index, or the value of y when $x = 1$. To obtain a straight stream we take common logarithms of both the variables in the parabolic equation given above, and we get: $\log y = \log b + k \log x$. In short, we work entirely with logarithms of the body and organ weights instead of with the observed weights. This relative growth function has been found extremely useful in studying the relation of a part to the whole in various animals, and by a number of biologists since Snell (1891). Their work need not be listed here since it can be easily traced in Huxley's book (1932) and in the journal GROWTH.

Its application to organ-weights, so much more important for experimental work in the rat, has received small attention. Some data

and brief treatment by Walter and Addis (1939) on several organs in albino rats and by Gray and Mahan (1943) on the heart in man and in rodents (1945) have led to the present extended study of the logarithmic growth law.

The law is useful for the heart, kidneys, and liver, which will be presented in this paper. Some organs tend to a sigmoid form of growth and the observations on them have to be smoothed by more complicated equations. These will be touched on in this paper, but for the most part deferred to an ensuing paper, mainly on the suprarenals and gonads.

An amazing number of authors have been quite content to evaluate the constants for the rate or slope k, and the initial growth index b, from two animals only, namely a pair of values (body-weight and organ weight) for a small animal, and another pair for a large animal. The crudity of this approximation to the postulated straight line law is apparent from any series of animals reported for a species; considerable variability between individuals within the species is conspicuous, and consequently manifests the unreliability of those equations in the literature based on two individuals taken at random. In fact the crudity is such as to hamper comparisons between the constants obtained for varying groups, and as such comparisons are the essence of comparative anatomy and physiology, we have felt obliged to resort, despite the enormous increase in labor, to the following method of treatment of the data. It is now seen to be fortunate that a number of editors have had a sufficiently long view to give expensive space in their journals to detailed original observations.

Much more laborious but much more reliable is the utilization of all the animals reported by an author, or, when they are too numerous, use of organ means for several (say 10-20) body-weight classes. Our procedure, therefore, has been to plot the logarithm of body-weight as X and the logarithm of organ-weight as Y. Through most of our work the units used are logarithms, not grams. Two lines were pencilled to indicate the approximate borders of the stream of observations, and square brackets used to mark points which lay outside the stream. Such points are (a) frequently due to the youth of the animals concerned, because in youth the organ-body relationship is often distinctly different from that pertaining to most of the growth period. Occasionally (b) they arise because increase in body-weight does not always cease when the rats have reached maturity but may con-

tinue thereafter provided they remain healthy. This is not true growth but an increase of weight that is due mainly to fat deposition. Rarely is it a consequence of presumptive errors in the record. While such omissions are admittedly arbitrary, they will not be individually discussed, because it is reasonable to assume that the true tendency of the lines desired is approximated more closely by use of the data after the exclusions, and also because the outlying exclusions amount to a minute percentage of the observations plotted.

Material and Methods

The colony came from the Slonaker strain, from which also the Wistar Institute colony was derived. For about 17 years prior to 1941, when we began the consolidation of the observations, the colony had passed through about 60 generations, living under conditions closely similar to the following. The food was given ad libitum and contained 17 per cent of protein. It was an air-dry mixture containing 10 per cent unpurified casein, 73 per cent corn meal, 10 per cent linseed meal, 2 per cent alfalfa, 3 per cent sardine oil, 1.5 per cent bone ash, and 0.5 per cent sodium chloride. From the viewpoint of uniformity, a special advantage here was that the temperature of the room in which the rats were kept averaged 20° C., and was regulated to prevent any marked fall, so that it was rarely less than 18° C., though on a few warm days it occasionally rose for a few hours appreciably above 20° C. in spite of a fan that kept a current of air constantly moving through the room. The rats were housed in groups of six in cages 17×12×12 inches.

There were several series of animals, studied for various purposes. They will be referred to as follows:

Compensatory Hypertrophy Series. This is much the largest series. They were all controls for experiments on the compensatory hypertrophy of the testicle, suprarenal, ovary, and kidney, and had all undergone a sham operation in which these organs were exposed but not removed. This operation required no more than a minute of time and had no appreciable effect on the increase of body weight in growth.

Sunlight Series. The compensatory hypertrophy series was also subdivided according to the days of outdoor sunlight expressed as a percent of life-span. For convenience in study, these percentages were grouped into four categories of equal range: 39.6 - 45.1, 45.2 - 50.7, 50.8 - 56.2, 56.3 - 61.7. The motive was to examine the effect of season.

A curve of "sunlight hours" was obtained from the meteorological people during the various months of the year. The organ weights were then arranged in the above categories.

Liver Protein Series. This was the control series on stock diet, and untreated, for experiments on the effect on organ weight of starvation, etc.

Dark and Light Series. These were small series in which we observed the effect on organ weight of keeping rats in the dark constantly, or constantly in electric light.

Blinded and Not-blinded. These were small series in which we took the eyes out of baby rats.

Heart Weight (HW)

Material.—We have these series:

	M	F
Compensatory Hypertrophy	831	810
Liver-Protein Series	291	90
Dark and Light Series	247	268
All together	1369	1168

For each series and sub-series, the mean HW at kill, in mg., was plotted against mean BW at kill, in gm., the animals having been grouped by BW in classes of 20 gm. class interval, 20-39 g., 40-59, etc., . . . to 400-419 g. for males, and to 260-279 g. for females. It should be noted that constancy in the way in which the heart is removed is an important factor in determining the variability of the measurements. In the data here reported the hearts were removed by one of the authors (T.A.), in a relatively uniform manner. The apex of the heart was held by forceps and lifted upwards so that the inferior vena cava and the pulmonary veins were exposed to view while they were being severed by scissors. The superior vena cava and the aortic and pulmonary arteries were cut through just above the still beating left auricle.

Pooling of Series.—The poolability of the several sets of data was then considered. The Compensatory Hypertrophy Series was divided in 4 sub-series according to the number of hours of probable outdoor sunlight, assumed to be a measure of seasonal effect. Such differences as existed between the four sub-series means of HW for each BW-

class showed no meaningful tendency; i.e., the hours of daylight had no effect on HW, and hence the Dark and Light Series and the Compensatory Series can be treated as a whole. The Liver-Protein Series was not subdivided by daylight hours in studying HW. The Dark sub-series was next compared with the Light, by inspection of the row of heart means in successive body-weight groups of the lights as against the corresponding values for the darks. The body-weight groups were each of 5 gms. class interval, so that we had 30 classes. Fifteen of the classes showed larger HW for the lights, and fifteen showed larger HW for the darks, and there was no systematic order of the larger and smaller values. The conclusion was that the darks and lights had not been affected by the experimental set-up and accordingly they were treated as one series. This inspection was later carried out for kidney-weight and suprarenal weight and held true for these organs also. Finally sequences of means in each of the three main series were plotted; they lay so close together that, without going to the labor of computing standard errors, they were considered unaffected by the fact that they were observed at different dates, and therefore they were pooled, thus giving us data from 1369 males and 1168 females.

Basic Constants.—The fundamental data reduced from the pool, namely the summary of means and variabilities for heart weight, are put on record in Tables 1 and 2.

Prediction Equations.—For each BW-group, the logarithm of the mean BW and of the mean HW is plotted in Fig. 1. The points, omitting a few outliers at the extremes, which represent scanty data for the group, are obviously well fitted by straight lines.

Two phases are detectible with a break about the time of puberty, for males between the classes divided at 120 grams BW (log = 2.08 on the horizontal scale in the graph), and for females between the classes divided at 100 g. BW (log = 2.00 on the X-scale). The several equations are put on record below, together with the number of rats, N, and the number of points, n, used in computing.

Male, Phase I	Y = 0.987 + .806 X,	(N 510), n 5
II	Y = 1.175 + .720 X,	(N 855), n 14
Final, I + II	Y = 1.059 + .768 X,	(N 1365), n 19
Female, Phase I	Y = 1.026 + .788 X,	(N 396), n 4
II	Y = 1.129 + .738 X,	(N 767), n 7
Final I + II	Y = 1.060 + .768 X,	(N 1163), n 11

These final equations differ from those reported in the 1945 paper

TABLE 1

HEART WEIGHT, MALE

Albino rats on stock diet. Basic constants in milligrams. The standard deviation was calculated with Sheppard's correction for coarse grouping.

Body Weight in grams		No. of rats	Heart Weight in mg.			
20 g. class interval	Mid point	f	Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
20-39	29.5	24	181	15.4	3.14	8.5
40-59	49.5	122	229	25.0	2.27	11.0
60-79	69.5	94	298	30.1	3.10	10.1
80-99	89.5	133	360	29.6	2.56	8.2
100-119	109.5	137	427	33.4	2.85	7.8
120-139	129.5	77	493	41.3	4.70	8.4
140-159	149.5	98	553	33.4	3.37	6.0
160-179	169.5	89	602	54.4	5.77	9.0
180-199	189.5	100	650	48.0	4.80	7.4
200-219	209.5	66	701	60.1	7.39	8.6
220-239	229.5	93	764	60.5	6.27	7.9
240-259	249.5	81	807	59.5	6.61	7.4
260-279	269.5	44	847	55.5	8.36	6.6
280-299	289.5	51	866	58.4	8.18	6.8
300-319	309.5	47	933	63.6	9.28	6.8
320-339	329.5	45	969	76.9	11.47	7.9
340-359	349.5	34	999	76.8	13.17	7.7
360-379	369.5	21	1081	65.8	14.36	6.1
380-399	389.5	9	1065	102.6	34.20	9.6
400-419	409.5	3	1143	83.9	48.45	7.3
		1368				

TABLE 2

HEART WEIGHT, FEMALE

Albino rats on stock diet. Basic constants in milligrams. The standard deviation was calculated with Sheppard's correction for coarse grouping.

Body Weight in grams		No. of rats	Heart Weight in mg.			
20 g. class interval	Mid point	f	Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
20-39	29.5	12	184	9.8	2.82	5.3
40-59	49.5	86	236	24.3	2.62	10.3
60-79	69.5	119	294	41.3	3.79	14.0
80-99	89.5	179	366	34.1	2.55	9.3
100-119	109.5	94	424	35.0	3.61	8.3
120-139	129.5	93	490	47.1	4.88	9.6
140-159	149.5	196	539	41.8	2.99	7.8
160-179	169.5	158	589	45.6	3.63	7.8
180-199	189.5	138	641	51.2	4.36	8.0
200-219	209.5	67	697	48.7	5.95	7.0
220-239	229.5	21	727	52.3	11.41	7.2
240-259	249.5	4	758	25.4	12.68	3.3
260-279	269.5	1	827	—	—	—
		1168				

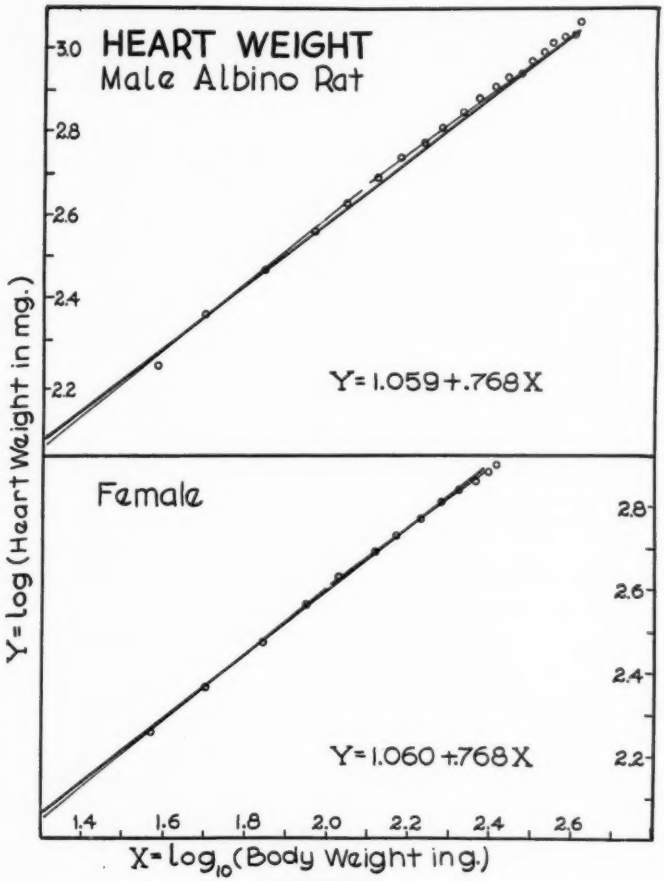


FIGURE 1

on the smaller numbers 1096 M and 900 F; but the lines when drawn practically coincide, hence the differences are negligible. Also it may be remarked that the break is trivial; hence the final overall line for given sex may be used. We have, however, solved the equations for the two phases before and after the break, and tabulated the predicted heart weights. The males are given in Table 3 and the females in Table 4.

TABLE 3

STANDARDS FOR HEART-WEIGHT IN MALE ALBINO RAT (ADDIS AND GRAY)

Phase I ; for BW 20-119 g.: log (HW in mg.) = 0.9872 + 0.8058 log (BW in g.)
Phase II; for BW 120-400 g.: log (HW in mg.) = 1.1754 + 0.7201 log (BW in g.)
Eg., for BW = 24 g., predicted HW is 126 mg.

Body Weight in g.	0	1	2	3	4	5	6	7	8	9
2	108	113	117	122	126	130	134	138	142	146
3	150	154	158	162	166	170	174	178	182	186
4	190	194	197	201	205	209	212	216	220	224
5	227	231	234	238	242	245	249	252	256	260
6	263	267	270	274	277	280	284	288	291	294
7	298	301	305	308	312	315	318	322	325	328
8	332	335	338	342	345	348	352	355	358	361
9	365	368	371	374	378	381	384	387	391	394
10	397	400	403	406	410	413	416	419	422	426
11	429	432	435	438	441	444	448	451	454	457
12	470	473	476	479	482	485	487	490	493	496
13	498	501	504	507	509	512	515	518	520	523
14	526	528	531	534	537	539	542	545	547	550
15	553	555	558	560	563	566	568	571	574	576
16	579	581	584	587	589	592	594	597	600	602
17	605	607	610	612	615	617	620	623	625	628
18	630	633	635	638	640	643	645	648	650	653
19	655	658	660	663	665	667	670	672	675	677
20	680	682	685	687	689	692	694	697	699	702
21	704	706	709	711	714	716	719	721	723	726
22	728	730	733	735	738	740	742	745	747	749
23	752	754	756	759	761	764	766	768	770	773
24	775	778	780	782	784	787	789	791	794	796
25	798	801	803	805	807	810	812	814	817	819
26	821	823	826	828	830	832	835	837	839	842
27	844	846	848	851	853	855	857	859	862	864
28	866	868	871	873	875	877	880	882	884	886
29	888	891	893	895	897	899	902	904	906	908
30	910	912	914	917	919	921	923	926	928	930
31	932	934	936	938	941	943	945	947	949	952
32	954	956	958	960	962	964	966	968	971	973
33	975	977	979	981	983	986	988	990	992	994
34	996	998	1000	1002	1005	1007	1009	1011	1013	1015
35	1017	1019	1021	1023	1025	1028	1030	1032	1034	1036
36	1038	1040	1042	1044	1046	1048	1050	1052	1054	1057
37	1059	1061	1063	1065	1067	1069	1071	1073	1075	1077
38	1079	1031	1083	1085	1087	1089	1091	1093	1096	1098
39	1100	1102	1104	1106	1108	1110	1112	1114	1116	1118
40	1120									

TABLE 4

STANDARDS FOR HEART-WEIGHT IN FEMALE ALBINO RAT (ADDIS AND GRAY)

Phase I ; for BW 20-99 g.: log (HW in mg.) = 1.0261 + 0.7875 (log BW in g.)
Phase II; for BW 100-240 g.: log (HW in mg.) = 1.1286 + 0.7375 (log BW in g.)
Eg., for BW = 24 g., predicted HW is 130 mg.

Body Weight in g.	0	1	2	3	4	5	6	7	8	9
2	112	117	121	125	130	134	138	142	146	151
3	155	159	163	167	171	175	178	182	186	190
4	194	198	202	205	209	213	217	220	224	228
5	231	235	238	242	246	249	253	256	260	264
6	267	270	274	277	281	284	288	291	295	298
7	301	305	308	312	315	318	322	325	328	332
8	335	338	341	345	348	351	354	358	361	364
9	367	370	374	377	380	383	386	390	393	396
10	401	404	407	410	413	416	419	422	425	428
11	431	434	436	439	442	445	448	451	454	456
12	459	462	465	468	470	473	476	479	482	484
13	487	490	493	496	498	501	504	506	509	512
14	514	517	520	522	525	528	531	533	536	539
15	541	544	547	549	552	554	557	560	562	565
16	568	570	573	576	578	581	583	586	589	591
17	594	596	599	601	604	606	609	612	614	617
18	619	622	624	627	629	632	634	637	639	642
19	644	647	649	652	654	657	660	662	664	667
20	669	672	674	677	679	682	684	686	689	691
21	694	696	699	701	704	706	708	711	713	716
22	718	720	723	725	728	730	732	735	737	740
23	742	744	747	749	752	754	756	758	761	763
24	766									

Units Used.—The units used above and throughout this paper have been logarithms to base 10, in order to deal with straight-line equations. They can be converted to the less convenient but occasionally used form of a parabolic curvilinear equation; for example, the final formula for the 1365 male rats, would become

$$y = 11.44\, x^{0.768}$$

$$\text{HW in mg.} = 11.44\ (\text{BW in g.})^{.768}$$

Prediction Error.—Walter and Addis found for their male equation a percentage error running from +3.0 to —2.6, average 1.6%; and for their female equation from +1.8 to —1.0, average 0.7%. For the present equations the range was +2.3 to —1.2, and the average without regard to sign was 0.9%. This is practically as good;

and, since the present equations, which omit the former additive constant, are simpler, they are preferable.

Sex-difference.—In a previous paper on heart-weight in rodents (Gray 1945) it was shown by diagram and by computation that the effect of sex was negligible, at least by comparison with other effects such as colony or experimental treatment, which are our main concerns. The finding is therefore of some importance because it makes analysis of small samples much easier by pooling the sexes. Strictly speaking, the albino male had a heavier heart than the female of like body-weight by only 1 percent; this was true in most species, though in data available on brown rats the male heart was actually lighter.

Variability.—Biological variation of individual rats around any average is amazingly large, and is conveniently judged by the coefficient of variation (100 S.D./Mean). Comparisons are complicated by the preferences of different investigators for groups on different bases of reference, such as age or body-weight; and for different class-ranges of the basic trait elected, such as 1 day vs. 1 week, or 10 grams vs. 20 grams. For example, for heart weight in albino rats at his 6 set ages: Birth, 7 days, 21, 41, 70, and 150 days, Jackson reported the coefficient of variation as ranging from 18 to 34%, with an average of 24%. Now any grouping of animals which reveals a variability within the set group of more than say 10%, measured in terms of the coefficient of variation, seems to us a command for a new method of grouping.

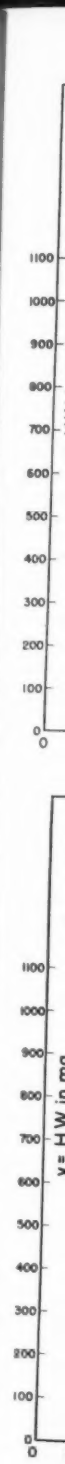
For that reason, among others, the base of grouping in this laboratory has been body weight, usually covering 20-grams to a class. For a first estimate of the variability of HW, we present the following. Our 1369 males fell into 18 such BW-classes. Taken by classes there were 24 rats in the first class, 122 in the second, and so on, as shown in Table 1. For the first class, we calculated beside the mean heart weight the variation of the 24 heart weights around that mean, finding a coefficient of variability of 8.5%; and so on for the other classes. The resulting 18 CV's ranged from 6.0 to 11.0%; omitting classes which contained 10 rats or less, these 18 values can be examined like any variable. Their mean was 7.9%. The further constants are shown in the adjoining text table. The item to keep in mind is that the coefficient of variation of HW's was approximately 8%, regardless of body weight.

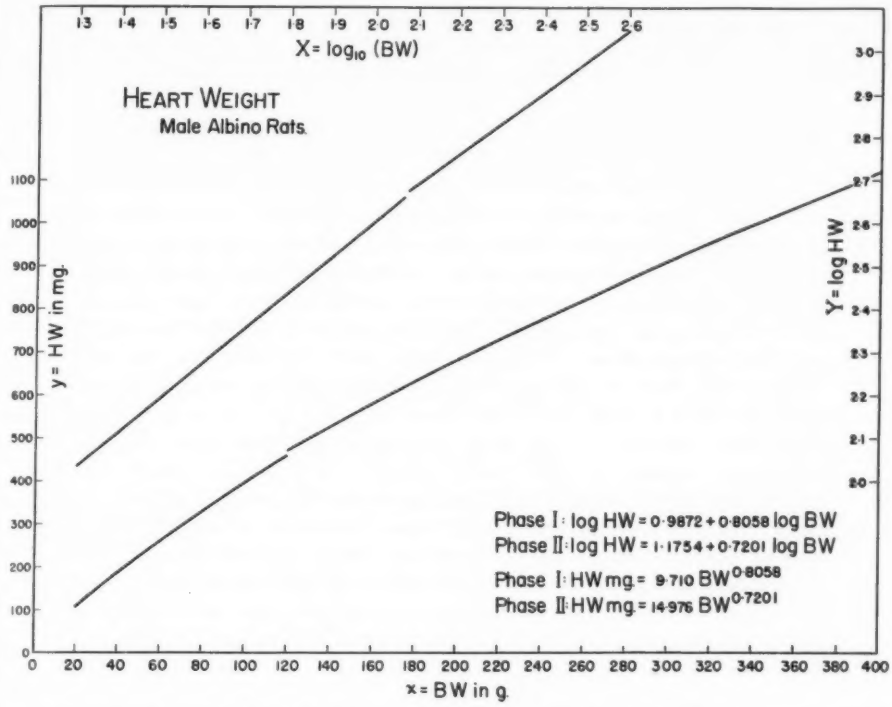
Groups			Coefficient of Variation, CV			
Sex	No. of rats	BW-classes	Range	Mean CV	Standard Deviation	Standard Error of Mean
Male	1369	18	6.0-11.0	7.9	1.29	.30
Female	1168	11	3.3-14.0	8.6	2.25	.68

A second estimate of variability of HW can be derived from a totally separate series (Laboratory Book 56, Albino males, starved 7 days). Their BW at kill ranged from 130 to 220, with a mean of 179 grams and a CV of 12%. Even without attempt to discount this variability in BW, which of course increases the HW variability, we find for HW these results: mean 644 g., standard deviation 67 g., CV 10%, which, remembering that BW was not eliminated, is not at all bad.

A third more precise and more reliable estimate of the variability of HW can be derived from a totally separate series of experiments which will be treated in another paper. From that series (Laboratory Book 108) we take the first ten experiments; each comprised of twelve rats. The 128 rats were initially selected with BW 148-152 grams, and within each experiment the 12 rats were treated alike, both as to operation, diet, and day of kill; furthermore, in order to eliminate BW at time of kill, the HW at kill was converted by formula, as described in the paper by Walter and Addis, to a HW corresponding to an assumed BW of 150 grams at kill; this value we term heart weight corrected, HWc. Let us examine this variable. We take the first experiment, and compute for our variable, namely, HWc, the conventional mean, standard deviation, and CV; the last named was 5.8%. We repeat for the next 9 experiments; securing CV ranging from 3.7 to 7.3. For the ten experiments these averaged as 5.8%, which is better than in preceding paragraph and indeed is a gratifyingly low variability.

Albino Rats Reported by Other Observers.—The colony, that is, strain plus environment in different laboratories, has a marked effect; Hatai's rats and Jackson's had larger hearts than ours. The differences were shown diagrammatically in a prior paper (1945, Fig. 2). The degree of difference from the present material may now be estimated by the following procedure. Take three convenient body weights, low, medium and high, say 20, 160, and 400 g. Substitute




X = log10 (BW)
HEART WEIGHT
Male Albino Rats.
y = HW in mg.
Y = log HW
Phase I : log HW = 0·9872 + 0·8058 log BW
Phase II : log HW = 1·1754 + 0·7201 log BW
Phase I : HW mg. = 9·710 BW^0·8058
Phase II : HW mg. = 14·976 BW^0·7201
x = BW in g.

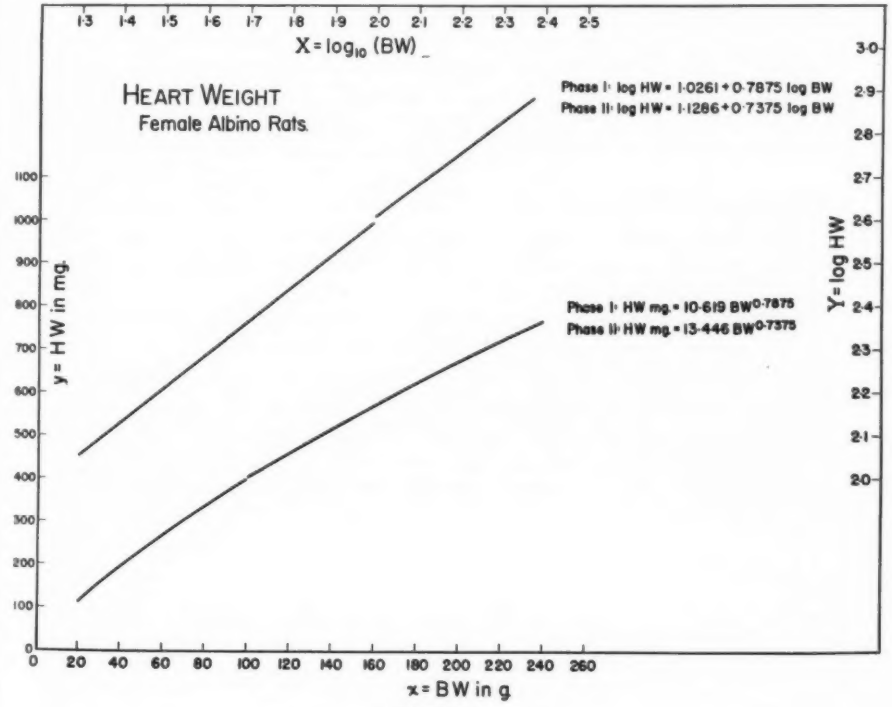

X = log10 (BW)
HEART WEIGHT
Female Albino Rats.
Phase I : log HW = 1·0261 + 0·7875 log BW
Phase II : log HW = 1·1286 + 0·7375 log BW
Phase I : HW mg. = 10·619 BW^0·7875
Phase II : HW mg. = 13·446 BW^0·7375
y = HW in mg.
Y = log HW
x = BW in g.

FIGURE 2

the corresponding logarithms 1.3010, 2.2041, 2.6021 for X in the four equations for the male albinos of Jackson, Hatai, Walter and Addis (re-calculated by us in 1945, Table 2; also Walter and Addis' equation and Gray's 1945 equation); and lastly in the final equation given earlier in this paper for 1369 males. Solve for Y, i.e., log HW, and re-convert the logs to milligrams. For each of the chosen body-weights, and working across the row, use the fifth or final value as base of reference, and express the divergence of each of the others as a percentage excess. Finally average down the columns; the outcome is that heart-weight predicted by the formula based on Jackson's data was 26% too big, by Hatai's data was 21% too large, by Walter and Addis' equation 5% too large, and by Gray's 1945 equation 0.7% too large. Hence the two earlier samples of material plainly had bigger hearts, while the latter two equations differed negligibly from the present series. This is natural since the latter two papers dealt with the same colony, with successively larger number of rats. The values, rounded off for simplicity, are shown in Table 5. It is a curious fact that inadequate nutrition—even starvation—has little effect on the relation between HW and BW. They both lose weight about to the same degree.

Comparisons of Species within the Family Muridae, and of the Muridae with Other Families within the Order Rodentia.—The details have already been published (1945, Table 3 and Figs. 3 and 5). The

TABLE 5
HEARTS LARGER IN EARLIER SERIES

BW in g	log BW=X	Jackson calc. by H.G. N=121	Hatai calc. by H.G. N=128	Walter & Addis 1939 N=795	Addis calc. by H.G. 1945 N=1096	Present Series, T.A.+H.G. N=1365
Low						
20	1.301	124	143	127	116	114
		+ 9%	+25%	+11%	+ 1.7%	
Average						
160	2.204	735	680	575	568	566
		+30%	+20%	+ 2%	+ 0.5%	
High						
400	2.602	1609	1345	1135	1144	1144
		+41%	+18%	− 1%	0 %	
Av. divergence for 3 points without sign		26%	21%	5%	0.7%	

differences worth emphasizing here are two: that hearts are larger in animals of the same species who live under conditions of specially active exercise, and in animals on high cholesterol diets.

Units Used.—The units used above and throughout this paper have been logarithms to base 10, in order to deal with straight-line equations. They can be converted to the less convenient but occasionally used form of a parabolic curvilinear equation; for example, the final formula for the 1365 male rats, would become

$$y = 11.44\, x^{0.768}$$
$$\text{HW in mg.} = 11.44\ (\text{BW in g.})^{.768}$$

Kidney Weight (KW)

Material.—For this organ there are observations listed in the preceding section on heart-weight. It will be remembered that there HW was found unaffected by the conditions of the several series, which were therefore all pooled to secure a final standard for each sex. Here, however, we found that the Dark-Light conditions affected KW, and therefore this series was omitted. The sunlight-subsets within the Compensatory Hypertrophy (CH) series were carefully compared and revealed nothing significant; likewise the line computed for the Liver-Protein (LP) series, though slightly below the CH just mentioned, did not seem worth separation; accordingly, we take the CH-LP series together to obtain standards for kidney-weight (KW): 1122 male albinos and 900 females.

Basic Constants.—This pool was reduced to the fundamental means and variabilities of kidney-weight (KW), for each of the body-weight (BW) classes, in Tables 6 and 7.

Prediction Equations.—As before, for each BW-class, the logarithm of mean BW as X and the logarithm of mean kidney-weight (both kidneys together) was plotted in Fig. 3. The points, omitting a few outlying values at the extreme (the males BW-class 400-419 g., and the females BW-classes 20-39 g., 240-259 g., 260-279 g.), each of which represented scanty data, are well fitted by straight lines.

Two phases are to be seen with a break at puberty. This fell approximately for males between the classes demarcated at 100 grams BW or 2.00 on the logarithmic horizontal scale in Fig. 3, i.e., somewhat earlier in growth than was noted in the preceding section on heart-weight, i.e., 120 g., log = 2.08, and for females at 100 g. or

TABLE 6
2 KIDNEYS WEIGHT, MALE

Albino rats on stock diet. Basic constants in milligrams. The standard deviation was calculated with Sheppard's correction for coarse grouping.

Body Weight in grams			2 Kidneys Weight in mg.			
20 g. class interval	Mid point	No. of rats f	Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
20-39	29.5	24	396	27	5.6	6.9
40-59	49.5	114	488	60	5.6	12.3
60-79	69.5	65	627	70	8.7	11.2
80-99	89.5	51	735	52	7.3	7.1
100-119	109.5	47	893	84	12.2	9.4
120-139	129.5	46	1012	109	16.1	10.8
140-159	149.5	91	1121	106	11.1	9.5
160-179	169.5	89	1236	119	12.6	9.6
180-199	189.5	100	1317	120	12.0	9.1
200-219	209.5	66	1399	139	17.1	9.9
220-239	229.5	93	1476	114	11.8	7.7
240-259	249.5	81	1562	151	16.8	9.6
260-279	269.5	44	1652	116	17.5	7.0
280-299	289.5	51	1747	140	19.6	8.0
300-319	309.5	47	1869	178	26.0	9.5
320-339	329.5	45	1946	202	30.1	10.4
340-359	349.5	34	2008	176	30.2	8.8
360-379	369.5	21	2157	165	36.0	7.6
380-399	389.5	10	2108	226	47.6	7.2
400-419	409.5	3	2389	106	61.1	4.4
		1122				

TABLE 7
2 KIDNEYS WEIGHT, FEMALE

Albino rats on stock diet. Basic constants in milligrams. The standard deviation was calculated with Sheppard's correction for coarse grouping.

Body Weight in grams			2 Kidneys Weight in mg.			
20 g. class interval	Mid point	No. of rats f	Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
20-39	29.5	12	364	34	9.8	9.3
40-59	49.5	81	493	58	6.4	11.7
60-79	69.5	75	618	63	7.3	10.2
80-99	89.5	29	770	69	12.8	8.9
100-119	109.5	29	880	72	13.3	8.1
120-139	129.5	90	981	76	8.0	7.7
140-159	149.5	195	1057	83	6.0	7.8
160-179	169.5	158	1131	86	6.9	7.6
180-199	189.5	138	1216	84	7.1	6.9
200-219	209.5	67	1295	97	11.8	7.4
220-239	229.5	21	1372	55	12.0	4.0
240-259	249.5	4	1499	135	67.5	9.0
260-279	269.5	1	1614	—	—	—
		900				

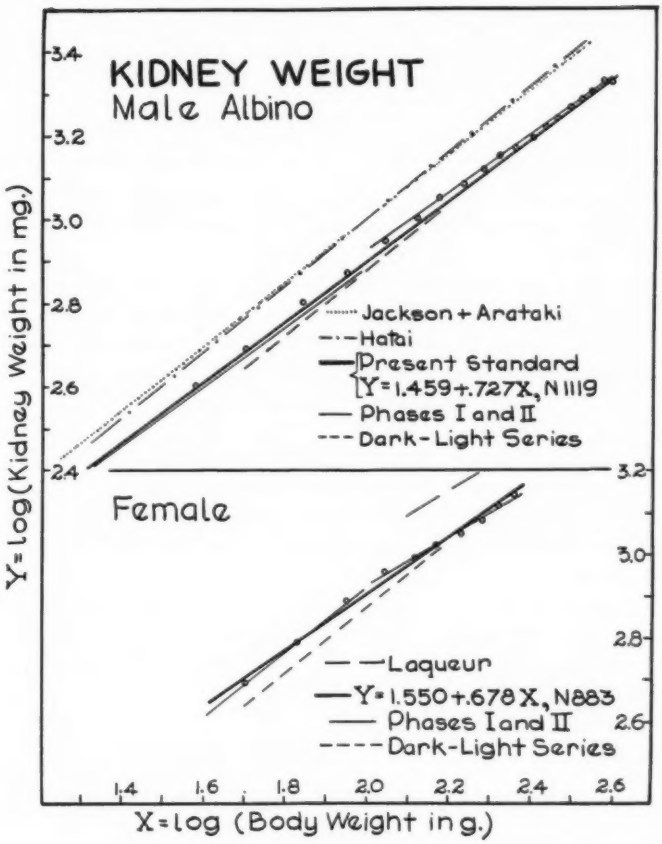


FIGURE 3

log 2.00, i.e., the same as noted for heart-weight. The break is rather more marked than for heart-weight, so that the equation for either the earlier or the later phase may be wanted when the greatest accuracy is needed; but the break was not so marked but that a single overall line seems suitable for most purposes The several equations are put on record, together with the number of rats, N, and the number of points, n, used in calculating.

Male

Phase I $Y = 1.472 + .717\ X$, (N 254), n 4, Range BW: 80-99 g.
II $Y = 1.533 + .696\ X$, (N 865), n 15, Range BW: 100-399 g.
I + II $Y = 1.459 + .727\ X$, (N 1119), n 19, Range BW: 80-399 g.

Female

Phase I $Y = 1.320 + .805\ X$, (N 185), n 3, Range BW: 40-99 g.
II $Y = 1.731 + .595\ X$, (N 698), n 7, Range BW: 100-239 g.
I + II $Y = 1.550 + .678\ X$, (N 883), n 10, Range BW: 40-239 g.

For the convenience of laboratory work, the phase equations have been solved and the 2 KW predicted for any BW are presented in Tables 8 and 9.

Dark and Light Series.—This material in Fig. 3 shows smaller kidneys than the standard. No explanation is apparent for the finding. The table of these means is accordingly not published, but merely the equations for the dashed lines in the diagram:

Male $Y = 1.325 + .778\ X$, (N 247), n 6
Female $Y = 1.274 + .799\ X$, (N 268), n 6

Variability.—Analogous to the discussion under HW, we present here estimates for variation in KW. Jackson, who grouped his rats at several ages, reported coefficients of variability which averaged 22%, i.e., about the same as for HW. This instability is too great to be of use.

Our own first estimate, comparable to the 8% for HW in the preceding section, yields a CV for 2 KW of about 9%. If one wishes more detail on the values of CV for each BW-class, each regarded as the variate, see the adjoining text-table.

			Coefficient of Variation, CV			
Sex	No. of rats	BW-classes	Range	Mean CV	Standard deviation	Standard Error of Mean
Male	1109	18	6.9-12.3	9.1	1.52	2.00
Female	895	11	14.0-11.7	8.2	0.36	0.59

Another estimate, comparable to the third estimate for HW-corrected, which was 5.8%, comes out for 2 KW-corrected (to 150 grams BW) as 6.9%.

The Minimal Variability.—The 39 individual class values for CV which yield the means shown, need not be itemized. But we have been interested in examining their magnitude in order to estimate the earliest BW-class for which KW-variability becomes approximately

TABLE 8

STANDARDS FOR 2 KIDNEYS-WEIGHT IN MALE ALBINO RAT (ADDIS AND GRAY)

Phase I ; for BW 20-99 g.: log (2 KW in mg.) = 1.4722 + 0.7168 log (BW in g.)
Phase II; for BW 100-400 g.: log (2 KW in mg.) = 1.5328 + 0.6963 log (BW in g.)
Eg., for BW = 24 g., predicted 2 KW is 289 mg.

Body Weight in g.	0	1	2	3	4	5	6	7	8	9
2	254	263	272	281	289	298	306	315	323	331
3	340	348	356	364	372	379	387	395	402	410
4	417	425	432	440	447	454	461	469	476	483
5	490	497	504	511	518	524	531	538	545	552
6	558	565	572	578	585	591	598	604	610	617
7	623	630	636	642	649	655	661	667	674	680
8	686	692	698	704	710	716	722	728	734	740
9	746	752	758	764	770	776	782	788	793	799
10	842	848	854	860	865	871	877	883	889	894
11	900	906	911	917	923	928	934	940	945	951
12	956	962	967	973	978	984	989	995	1000	1006
13	1011	1016	1022	1027	1032	1038	1043	1049	1054	1059
14	1064	1070	1075	1080	1086	1091	1096	1101	1107	1112
15	1117	1122	1127	1132	1138	1143	1148	1153	1158	1163
16	1168	1173	1178	1184	1188	1193	1199	1204	1209	1214
17	1218	1224	1229	1233	1238	1243	1248	1253	1258	1263
18	1268	1273	1278	1283	1288	1293	1298	1302	1307	1312
19	1317	1322	1326	1331	1336	1341	1346	1350	1355	1360
20	1365	1369	1374	1379	1384	1388	1393	1398	1402	1407
21	1412	1416	1421	1426	1430	1435	1440	1444	1449	1454
22	1458	1463	1468	1472	1476	1481	1486	1490	1495	1499
23	1504	1509	1513	1518	1522	1527	1531	1536	1540	1545
24	1549	1554	1558	1563	1567	1572	1576	1580	1585	1590
25	1594	1598	1603	1607	1612	1616	1620	1625	1629	1634
26	1638	1642	1647	1651	1656	1660	1665	1669	1673	1678
27	1682	1686	1690	1695	1699	1703	1708	1712	1716	1721
28	1725	1729	1733	1738	1742	1746	1751	1755	1759	1763
29	1768	1772	1776	1780	1784	1789	1793	1797	1801	1806
30	1810	1814	1818	1822	1826	1831	1835	1839	1843	1848
31	1852	1856	1860	1864	1868	1872	1876	1881	1884	1889
32	1893	1897	1902	1906	1909	1913	1917	1921	1926	1930
33	1934	1938	1942	1946	1950	1954	1958	1962	1966	1971
34	1975	1979	1982	1987	1991	1995	1999	2003	2007	2010
35	2015	2019	2023	2027	2031	2035	2038	2043	2047	2051
36	2055	2059	2063	2067	2071	2074	2079	2083	2086	2090
37	2094	2098	2102	2106	2110	2114	2118	2122	2126	2130
38	2134	2138	2141	2145	2149	2153	2157	2161	2165	2168
39	2173	2176	2180	2184	2188	2192	2196	2199	2203	2208
40	2211									

TABLE 9

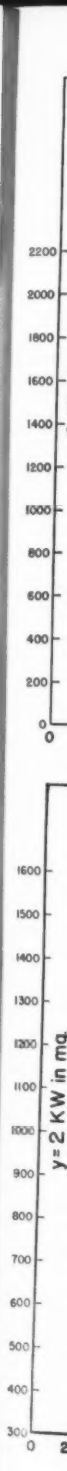
STANDARDS FOR 2 KIDNEYS-WEIGHT IN FEMALE ALBINO RAT (ADDIS AND GRAY)

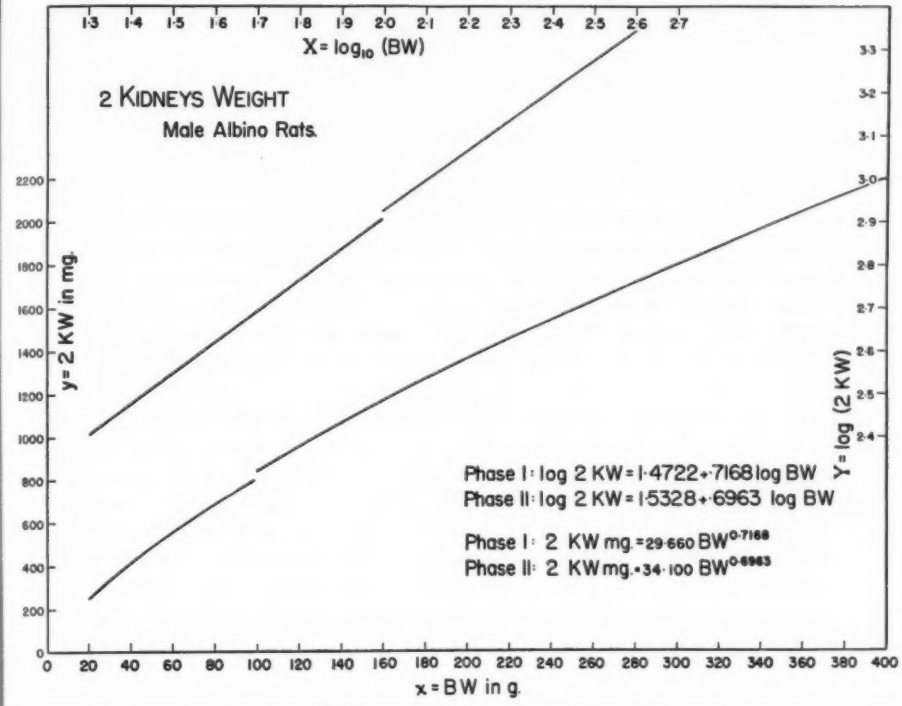
Phase I ; for BW 40-99 g.: log (2 KW in mg.) = 1.3201 + 0.8046 log (BW in g.)
Phase II; for BW 100-240 g.: log (2 KW in mg.) = 1.7313 + 0.5951 log (BW in g.)
Eg., for BW = 44 g., predicted 2 KW is 439 mg.

Body Weight in g.	0	1	2	3	4	5	6	7	8	9
4	406	415	423	431	439	447	455	463	471	479
5	486	494	502	510	518	525	533	541	548	556
6	563	571	578	586	594	601	608	616	623	630
7	638	645	652	660	667	674	681	689	696	703
8	710	717	724	732	739	746	753	760	767	774
9	780	788	795	802	808	816	822	829	836	843
10	835	840	844	849	854	859	864	869	874	879
11	883	888	893	898	902	907	912	916	921	926
12	930	935	940	944	949	953	958	962	967	971
13	976	980	985	989	993	998	1002	1007	1011	1015
14	1020	1024	1028	1032	1037	1041	1045	1050	1054	1058
15	1062	1067	1071	1075	1079	1083	1087	1092	1096	1100
16	1104	1108	1112	1116	1120	1124	1128	1132	1137	1140
17	1144	1149	1152	1156	1160	1164	1168	1172	1176	1180
18	1184	1188	1192	1196	1200	1204	1208	1211	1215	1219
19	1223	1227	1231	1234	1238	1242	1246	1250	1253	1257
20	1261	1264	1268	1272	1276	1280	1283	1287	1291	1294
21	1298	1302	1305	1309	1312	1316	1320	1324	1327	1331
22	1334	1338	1342	1345	1349	1352	1356	1360	1363	1366
23	1370	1374	1377	1381	1384	1388	1391	1395	1398	1402
24	1405									

constant, as compared with the run of succeeding values; at such a BW we may then say that the animals have reached an area of reasonably minimal fluctuation in respect to total weight; this area seems then desirable as a guide to selecting rats for uniformity of sample in preparation for an experiment on KW. Accordingly, we note that the CV in successive classes became practically uniform for males with BW 140-160 g. and thereafter; for females with BW 100-120 g. and thereafter. Theoretically, therefore, it seems logical, when picking rats for uniformity, to take males of 150 grams and females of 110 grams; each within a range of 1 gram (150 = 149-151). In fact this procedure has proved practical in the laboratory.

Asymmetry.—The rat's right kidney is larger than the left. According to MacKay (1937)'s data, the ratio R/L is for males 103.0 and for females 103.9; i.e., let us say the right was about 3 percent heavier. The present larger material yields a value nearer 5 percent.




X = log10 (BW)
2 KIDNEYS WEIGHT
Male Albino Rats.
y = 2 KW in mg.
Y = log (2 KW)
Phase I : log 2 KW = 1·4722 + ·7168 log BW
Phase II : log 2 KW = 1·5328 + ·6963 log BW
Phase I : 2 KW mg. = 29·660 BW^0·7168
Phase II : 2 KW mg. = 34·100 BW^0·6963
x = BW in g.

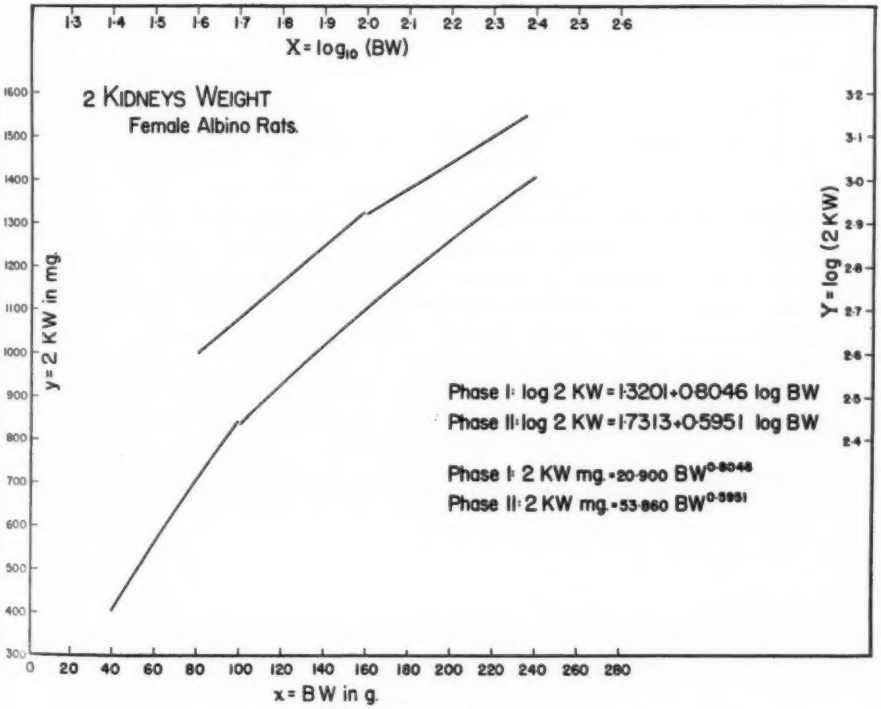

X = log10 (BW)
2 KIDNEYS WEIGHT
Female Albino Rats.
y = 2 KW in mg.
Y = log (2 KW)
Phase I : log 2 KW = 1·3201 + 0·8046 log BW
Phase II : log 2 KW = 1·7313 + 0·5951 log BW
Phase I : 2 KW mg. = 20·900 BW^0·8046
Phase II : 2 KW mg. = 53·860 BW^0·5951
x = BW in g.

FIGURE 4

The explanation suggested by the MacKays was that the shorter right renal artery gives greater blood supply.

Our quantitative comparison was arrived at by the following procedure: a table was drawn up listing our usual body-weight classes, the mean right KW, the mean left KW, and the ratio between the two sides. This may be called the asymmetry index or R/L index. Its values may be handled like any variate in conventional manner; the classes with frequencies, f, of 10 or fewer rats have been omitted because such small numbers yielded such wide fluctuations of the index (3 male classes and 4 female classes); and for each remaining class the frequency less one, f—1, was used as Fisher's degrees of freedom in computing the formulas. The resulting constants are shown in the accompanying text-table. The difference between the sex means in relation to the standard error of that difference, yields P 0.10, meaning the probability that ten out of a hundred such experiments would be expected to show such a difference. This probability, at the so-called ten-percent level, is not conclusive, but would usually be taken as suggestive that there really exists slightly greater bilateral asymmetry in male kidneys than in females.

Sex	Rats N	Classes f	Mean R/L Index	Standard Deviation	Standard Error of Mean
Male	829	17	106.0	4.13	1.00
Female	810	19	104.4	1.45	0.33

Effect of Colony; Albino Rats Reported by Several Observers.—As with heart-weight, the kidneys were notably larger in other colonies. To illustrate, the males are shown at top of Fig. 3. And, when the several colonies are treated in the same way as in Table 3 for heart, the kidneys in Jackson's and Arataki's rats (dotted line) were 27% larger than our present standard; Hatai's (dash-dot) 26% larger; and Walter and Addis' equation 2% larger. These figures parallel those stated in the section on heart-weight, and confirm the importance of testing any given colony studied, before choosing a standard.

The females are also shown in Fig. 3. For 2 KW we compute on the 123 females of Jackson (1913) and Arataki (1926) the equation: $Y = 1.371 + .814\,X$, N 123 females, 16-245 g. BW; on the 115 females of Laqueur's $Y = 1.642 + .683\,X$.

Then from the several equations for the several authors, we calculate as in the heart section, the predicted KW at the three BW chosen,

at the bottom, middle and top of the range of Laqueur's material; then express those predicted values in percent deviation from our equation for the corresponding Phase II; and finally average the three percentages for each sample. We find the Jackson-Arataki sample 36%, and Laqueur's sample 28% larger than ours.

Comparison of Albino Rat with Other Species of Muridae.—In this comparison the kidneys show more marked differences between species than did the heart. In Fig. 5, the solid dots represent the centre of

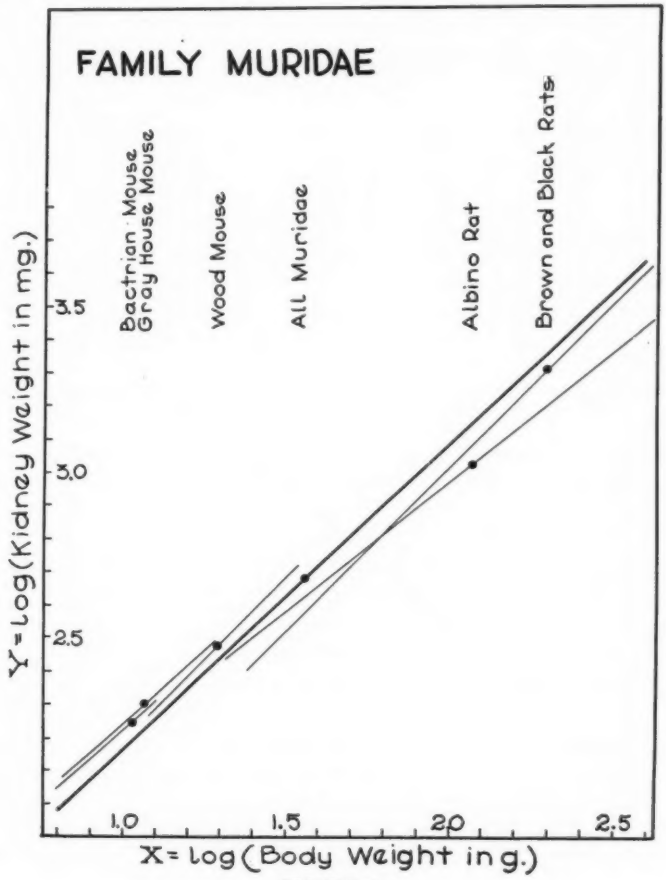


FIGURE 5

the distribution (mean X, mean Y) for pooled sexes in each species. It is seen that the magnitude of the kidneys relative to body-weight follows approximately this sequence, largest first:

Gray house mouse:	Y = 1.343 +	.887 X,	N	38
Bactrian mouse:	= 1.341 +	.877	N	11
Wood mouse:	= 1.143 +	1.028	N	38
Whole family Muridae:	= 1.247 +	.915	N	2654
Albino rat (ours):	= 1.059 +	.768	N	2528
Brown and black rat:	= 1.001 +	1.005	N	39

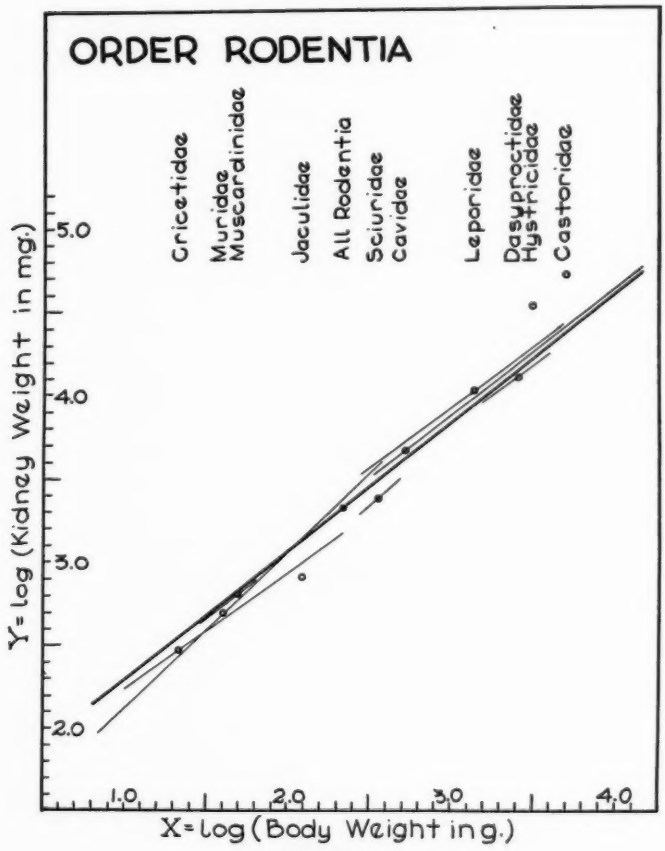


FIGURE 6

Comparison of Muridae vs. Other Families within Rodentia.—Now let us extend the comparison to larger groups. Males and females are treated together. The order of relative size of the kidneys approximates the following sequence, from small to large (Fig. 6).

Cricetidae (hamsters, voles):	Y =	1.545 +	.693 X,	N	48
Muridae (rats, mice):	=	1.247 +	.915	N	2654
Muscardinidae (dormice):	=	1.514 +	.755	N	24
Jaculidae (jerboas):	=	....	....	N	1
All Rodentia:	=	1.534 +	.759	N	2980
Sciuridae (squirrels):	=	1.267 +	.830	N	31
Cavidae (guinea-pigs):	=	1.737 +	.721	N	145
Leporidae (rabbits, hares):	=	1.904 +	.670	N	68
Dasyproctidae (agoutis):	=	1.673 +	.733	N	10
Hystricidae (porcupines):	=	....	....	N	2
Castoridae (beavers):	=	....	....	N	2

In the family of the Leporidae, the domestic had kidneys slightly heavier than wild; this is the reverse of the striking findings for heart-weight.

Prediction Error.—Walter and Addis found for their male equation a percentage error running from +4.6 to —3.2, average 1.6%; and for their female equation, an error running from +5.1 to —1.6, average 0.7%. On applying their formulae to the present larger material, the errors are somewhat greater, respectively 2.1% for males, 1.7% for females. Now, for the simple equations without the additive constant, which we present as standards, the percentage prediction errors are: 1.6% for males, 1.8% for females.

Sex.—As for heart-weight, the effect of sex was negligible, at least by comparison with other effects such as colony or experimental treatment, which are our main concerns. The finding is therefore of some importance because it warrants pooling of the sexes when one wishes to compare growth in rats with growth in other animals.

Age.—Two phases are shown, for each sex, with the equations, in Fig. 4.

Liver Weight (LW)

Material.—For liver weight we have only the liver-protein series: 291 males and 90 females.

Basic Constants.—The summary of means and variabilities, for successive body-weight groups of 20 grams class-intervals for males, and 10 grams intervals for females, are put on record in Tables 10 and 11.

Sex Difference.—In a previous paper (Walter and Addis) the note

TABLE 10
LIVER WEIGHT, MALE
Albino rats on stock diet. Basic constants in milligrams. The standard deviation was calculated with Sheppard's correction for coarse grouping.

Male						
Body Weight in grams						
20 g. class interval	Mid point	No. of rats f	Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
20-39	29.5	17	1779	158	38	8.9
40-59	49.5	43	2133	364	56	17.1
60-79	69.5	0	no data			
80-99	89.5	0	no data			
100-119	109.5	0	no data			
120-139	129.5	6	6750	761	311	11.3
140-159	149.5	57	7100	928	123	13.1
160-179	169.5	18	7250	494	116	6.8
180-199	189.5	21	7750	793	173	10.2
200-219	209.5	11	7977	629	190	7.9
220-239	229.5	48	8520	978	141	11.5
240-259	249.5	30	8833	667	122	7.6
260-279	269.5	12	9374	816	236	8.7
280-299	289.5	4	10500	629	315	6.0
300-319	309.5	6	9666	789	322	8.2
320-339	329.5	2	10500	—	—	—
340-359	349.5	8	11062	912	322	8.2
360-379	369.5	6	11914	1549	632	13.0
380-399	389.5	1	11750	—	—	—
400-419	409.5	1	13250	—	—	—
		291				

was made that more extensive data might reveal a sex difference in liver weight, but our present observations on female rat livers indicate that, if present, any such difference must be small. A plot (not reproduced) of the present means shows that the females had larger livers than the males in the early part of life, i.e., that cycle ignored for the most part in this paper because such young animals are seldom used in experiments; this unexpected direction of the difference may have been due to seasonal effect, but the evidence is not sufficient to decide definitely. Through the main part of life the females had rather smaller livers than the males, relative to body-weight of course; this directional difference will be seen more markedly for other organs.

Effect of Estimated Sunlight.—For this comparison, only males are examined, since the numbers of females were rather small. Each of the four sub-series was fitted by a straight line, shown in Fig. 7. The third series was also fitted in two phases, divided by an apparent

TABLE 11
LIVER WEIGHT, FEMALE
Albino rats on stock diet. Basic constants in milligrams.

Female			
Body Weight in grams 10 g. class interval	Mid point	No. of rats f	Mean M
30-39	34.5	12	1708
40-49	44.5	10	2100
50-59	54.5	8	2750
60-69	64.5	No data	
70-79	74.5	No data	
80-89	84.5	No data	
90-99	94.5	No data	
100-109	104.5	No data	
110-119	114.5	No data	
120-129	124.5	No data	
130-139	134.5	1	6750
140-149	144.5	29	6491
150-159	154.5	24	6458
160-169	164.5	6	6833
		90	

break at BW 160 g., or 2.415 on log scale, presumably due to puberty; the second phase equation, but not the line, is recorded in the graph. The conclusion is that, corresponding to the seasons of greater outdoor sunlight, the lower the line, the *smaller the liver-weight* for any given body-weight. An exception is for the greatest exposure, the fourth group, which is out of order, being the highest line, i.e., the largest livers; for this exception we have no explanation. Furthermore, we may comment once for all on the like comparison for heart-weight, kidney-weight and suprarenal weight. Among these only the suprarenal and only for phase II showed the tendency described. All in all then the effect of outdoor sunlight was so slight that it may be regarded as an immaterial factor among experimental conditions.

Prediction Equation.—If the means for liver-weight are plotted against the mid-points of the body-weight groups, one notices a certain amount of irregularity, as is common in any plot with biological material. This is due to the considerable variability which is found between normal animals and which unfortunately is too often ignored. However, for our purposes it is useful to have a rule for smoothing the means. In this case the straight line appears to fit nearly as well as a more complicated curve. This is predicated, as said before, on

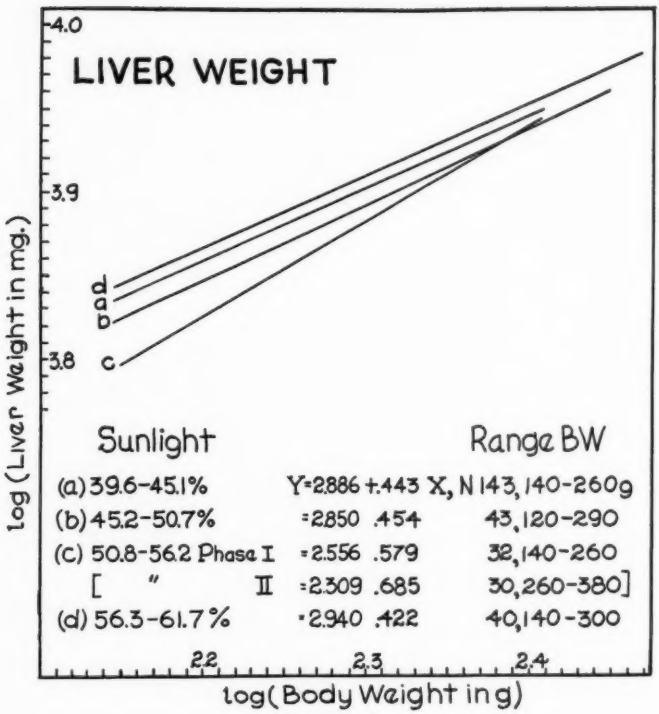


FIGURE 7

the conversion of the data from absolute units of weight to logarithms, and using these logarithms throughout as working units.

Inasmuch as there was so little difference between the sub-series according to sunlight, we now treat all of the males together. We take the logarithm of the mean liver-weight for each group and the logarithm of the mid-point of the body-weight class interval: for example, for the first class in Table 10, the log of the mean liver-weight 1779 mg. = 3.2502 and the log of the mid-point of the body-weight class interval 20-39 gms., i.e., 29.5 grams, is 1.4698; we call these Y and X respectively and proceed to make the usual extensions from them. After this has been done for each line, the sums of the extensions SX, SY, SX^2, SXY, enable us to calculate the equation for the

straight line. In this case the extensions were weighted by the frequencies, i.e., the number of rats in each class, in order to get slightly more accurate results. Omitting the first two classes, from 20-59 grams (at which early age or size the straight line does not hold), and the next three classes from 60-119 grams (on which we had no data), we then get for 230 rats the equation $Y = 2.745 + .5046\ X$, which covers the range 120-400 g. BW (Fig. 8),

where X = common logarithm of the mid-point of the body-weight class, and
Y = log of the mean liver-weight for that class.

For the female rats no equation was computed, because of the scanty number of observations, only 90 spread over all sizes.

Error of Prediction.—From this formula the predicted LW for each BW-class was computed, and converted from logs to milligrams. This prediction less the observed mean, divided by the observed, affords a convenient prediction-error. For the various classes it varied from 0.2 to 9.0%, and for the whole material, without regard to sign, averaged 3.3 percent.

Similarly for the formula of Walter and Addis (1939), applied to the data of Phase II, the percentage prediction error varied for the 14 BW-classes from —21 to +15%, with an average error of 8.7%. This makes clear the importance of discarding that earlier equation in favor of the present one which has a much smaller error.

However, the use of an equation with the extra constant can give an excellently small error. For example, on the present 230 male rats the equation was calculated:

$$LW = 5307 + 1.196\ BW^{1.4506}$$
$$\text{or } \log (LW—5307) = .0779 + 1.451 \log BW$$

and for this equation the prediction error varied from —7.1 to +3.5%, averaging without regard to sign, 2.1%. Yet, in general the extra labor does not seem worth while.

Variability.—Like the discussions under HW and KW, we present here some estimates for variability in liver weight. Jackson, grouping his rats at several ages, found coefficients of variability ranging from 19 to 33%; these may be averaged as 26%; i.e., much the same order as he found for HW and KW.

Our estimate, for rats grouped in BW-classes, comparable to our

8% for HW and 9% for KW, yields a CV for LW of 10%. The details, similar to the little text-tables previously used, are that there were 257 males, divided in 9 BW-classes; the CV in these ranged from 7 to 13%, averaged 10.2%, with standard deviation 3.3%.

Albino Rats Reported by Other Observers.—Hatai's male rats, as calculated by Brody, showed two phases. His second phase is sufficiently confirmed by our line, considering that it did not cover quite the same range. Our equation to represent Hatai's data on 129 rats is: $Y = 2.291 + .744\,X$. The notable feature is that Hatai's rats had much larger livers than ours for any given body-weight. This may be a result of a greater protein consumption in Hatai's rats. Alternatively, it may be a result of smaller body-weights in his colony, which is suggested by the finding, to be noticed later, that other organs also were larger for any given body-weight than in this colony. Laqueur's 115 female rats are fitted by the equation $Y = 2.112 + .800\,X$. The lines are to be seen in Fig. 8.

Other Species of Muridae Compared with Albino Rats.—Brown and black rats do not differ much from albinos, but the wood mouse has a larger liver relative to body-weight. The equations for male and female together, are:

Albino brown, and black rats,	N 273:	$Y = 1.473 + 1.101\,X$
Gray house mouse and wood mouse,	N 37:	$Y = 1.779 + .978\,X$

Other Families of Rodents Compared with Muridae.—No notable differences.

Muridae	N 669:	$Y = 1.680 + .986\,X$
Cricetidae	N 17:	$Y = 2.026 + .789\,X$
Muscardinidae	N 22:	$Y = 1.686 + .994\,X$
Sciuridae	N 28:	$Y = 0.753 + 1.284\,X$
Cavidae	N 8:	$Y = 2.119 + .816\,X$
Leporidae	N 37:	$Y = 1.618 + .956\,X$

Incidentally the wild rabbits and hares do not have larger livers than tame, a phenomenon noted for heart-weight and attributable to more active heart work.

Other Orders of Mammals Compared with Rodentia.—In general other orders do not differ strikingly from rodents. The exceptions, on the side of slightly bigger livers are the marsupials. And on the side of smaller livers are the chiroptera (bats), xenarthra (anteaters, sloths), carnivora (bears, weasels, dogs, cats, etc.), artiodactyla (cows, pigs, deer), perissodactyla (horses, zebras), cetacea (whales), and

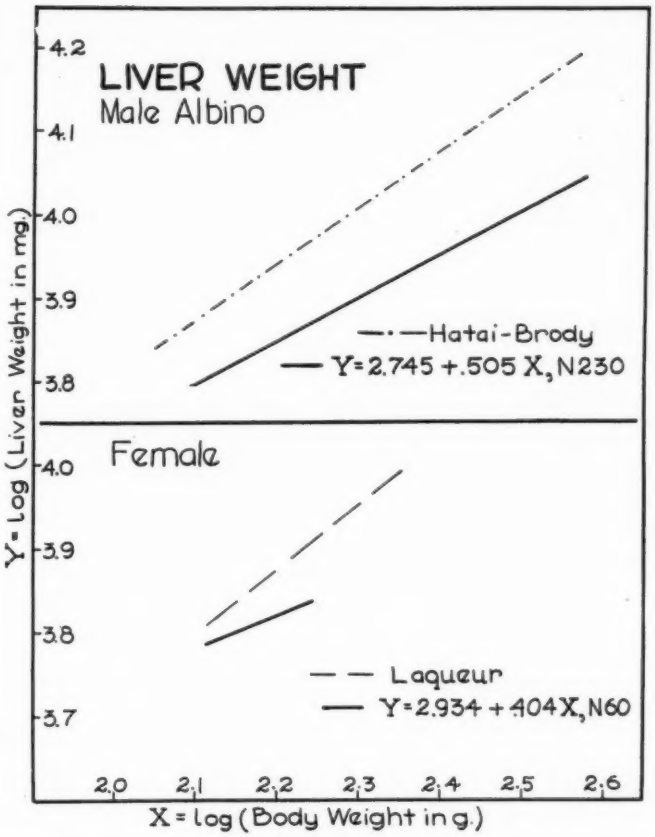


FIGURE 8

Laqueur's rats were kept on Tioga Chow. Its guaranteed analysis is protein 23.0%, fat 4.0%, fibre 4.0%, ash 12.5%, moisture 8.5%, and nitrogen-free extract 48%. Its ingredients are listed on the label as wheat cereals, meat and bone meal, soybean oil meal, fish meal, tomato pulp, wheat germ, cheese, corn gluten feed, alfalfa leaf meal, peanut meal, carotene in oil (Vitamin A concentrate), cod liver oil, yeast, minerals (sodium chloride, small amounts of iron oxide and traces of copper sulphate).

finally the proboscidea (elephants). Man and monkeys lie close to the rodents. We get the following equations, in order of average body-weight size for the orders:

Chiroptera	N	31:	Y =	1.674 +	.928	X
Insectivora	N	36:	Y =	1.445 +	1.082	X
Rodentia	N	781:	Y =	1.743 +	.950	X
Marsupialia	N	4:	Y =	1.373 +	1.130	X
Xenarthra	N	32:	Y =	1.420 +	.995	X
Carnivora	N	299:	Y =	1.808 +	.915	X
Monkeys	N	160:	Y =	1.753 +	.923	X
Homo	N	1889:	Y =	2.218 +	.837	X
Pinnipedia	N	10:	Y =	1.994 +	.912	X
Cetacea	N	8:	Y =	2.529 +	.761	X
Perissodactyla	N	165:	Y =	2.759 +	.714	X
Artiodactyla	N	67:	Y =	1.784 +	.883	X
Proboscidea	N	3:	Y =	2.284 +	.807	X

Summary

Experimental studies of the effects of treatment or other factors like diet, operation, etc., demand controls. At many times these must be run parallel to the experiment, but at other times the investigator can save time, labor, and cost by using standards, provided these be derived from carefully selected material. Such evidence on albino rats is here put on record, including diagrams to show the main principles, and tables furnishing the solved predicted organ weights for convenient laboratory use.

References

Brody, S. 1945. Bioenergetics and Growth. Reinhold Publishing Corp., New York. xii+1023.

Gray, E., & Mahan, E. 1943. Prediction of Heart Weight in Man. *Am. Jour. Physical Anthropology*, **1**, 271-287 and 4 figs.

Gray, H. 1945. Heart-Weight and Body-Weight in Rodents. *J. Mammalogy*, **26**, 285-299.

Hatai, S. 1913. On the Weights of the Abdominal and Thoracic Viscera. *Am. J. Anat.*, **15**, 87-119.

Huxley, J. S. 1932. Problems of Relative Growth. The Dial Press, New York. xix+276 pp., 105 illustr.

Jackson, C. M. 1913 Postnatal Growth and Variability in the Albino Rat. *Am. J. Anat.*, **15**, 1-68.

Kleiber, M. 1944. Energy Metabolism. *Ann. Rev. of Phys.*, **6**, 123-154.

Laqueur, G. Personal Communication.

MacKay, L. L., & MacKay, E. M. 1937. The Difference in the Weight of the Left and Right Kidneys. *Growth*, **1**, 309-311.

Snell, O. 1891. Die Abhängigkeit des Hirngewichtes von dem Körpergewicht. *Archiv für Psychiatrie und Nervenkrankheiten*, **23**, 436-446.

Walter, F., & Addis, T. 1939. Organ Work and Organ Weight. *J. Exper. Med.*, **69**, 467-483.

Growth, 1950, **14**, 81-92.

BODY SIZE AND SUPRARENAL WEIGHT

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Change in organ-weight as a criterion for severity of disease or for success of treatment has been commanding increasing attention in recent years. For example, the anatomical response of heart and kidneys to the functional load of increased work (Addis *et al.*, 1939); likewise the behavior of the liver under altered metabolic states, the endocrine glands under manifold physiological and pathological affects, and most lately the selective accumulation in sundry organs of radio-active isotopes such as radio-iodine in hyperthyroidism, radio-iron in anemias, radio-phosphorus in leukemia and polycythemia vera, radio-strontium for the pain of carcinomatous metastases in the skeleton (Lawrence, 1941; Rose, 1941; Levine *et al.*, 1941). For such a criterion the first requirement is knowledge of organ-weight in the normal animal. A recent statement reads that "Such data are not available." It would be more precise to state that such data are inconveniently scattered through anatomical, biological and zoological literature. A worse handicap is that such standards have been expressed in percentage of body-weight. But for most organs the relationship to body-weight is by no means a straight-line or percentage function. For many organs the practical reference is the logarithm of organ-weight to the logarithm of body-weight, and for other organs the relationship is more complicated but still with one or more logarithmic terms. Hence it follows that more accurate forecasting of organ-weight can be achieved in future by examining the data for various organs and species and for each such set of observations and computing a suitable type of formula. A vast collection of such material from the literature and from the laboratory experience of one of us (T.A.) is digested in the present study.

Genetic and Environmental Conditions.—With the passage of years, an experimenter finds that, after his primary hypotheses have

been tested, a large volume of observational data has accumulated which is appropriate for the determination of standards and even for the analysis of general laws of growth. The present accumulation has certain advantages.

(a) It represents a widespread strain of albino rats. Dr. Slonaker brought the originators of the colony to California in 1903. They were from a colony in the neurological department of the University of Chicago. From the same colony at a later date, the Wistar Institute colony was derived, and has been extensively studied by Donaldson. Genetically therefore this colony is representative of many other colonies of albino rats.

(b) This particular colony was started in 1924. Ever since then it has lived in the same laboratory and under the same environmental conditions. In this quarter century approximately 150 generations of rats have been born. This colony therefore would be expected to afford particularly stable standards for growth in the albino rat.

(c) The diet has remained nearly constant through the years. The temperature conditions have been uniform to a degree impracticable in most colonies. In many places, for example, the winter cold and more particularly the summer heat have caused such excessive variations from the optimum of 20° C., that for many months in every year the animals suffer sufficiently to qualify seriously the results observed. The operations have been done by T.A.

Prediction Methods.—For standards of prediction it is customary to utilize averages. But partly because of the variability of biological material which needs to be smoothed, and partly in order to obtain a formulation for forecasting and for comparison with other series, it is desirable to obtain mathematical equations to the data. In a recent paper, the heart, kidneys, and liver were conveniently represented by straight line equations when both variables were taken in logarithmic units; this means that the relative growth of organ and body was constant (Addis and Gray, 1950). We now have to consider the suprarenals and gonads.

It would be simplest to work in absolute units, i.e., body weight BW in grams, and organ weight in milligrams. But when the absolute organ weights are plotted against the absolute body weights, they form a curve or rather two curved phases, neither of which immediately suggests the fitting of any particular equation which might have a biological reason. This peculiarity might be anticipated embryologic-

ally. Furthermore, the male suprarenal growth is not sigmoid, but linear like heart and kidneys. The reason is presumed to be that the male adrenals are not subject to hormonal stimulation, but that the female are.

So one naturally tries logarithmic working units. That is, we take the common logarithm of BW and organ weight and work with those units. It is more laborious but often more rewarding. Furthermore, such log units may be more satisfactory from the point of view of comparing the formula finally chosen with the straight line formula in log units as proven for heart and kidneys. Another reason is that Hatai, who seems to have been the only observer to attempt to represent observed data for the suprarenals by some generalizing curve, found it desirable to introduce a logarithmic term. In order to represent the data smoothly, we have tested a number of formulae. Where convenient, the formulae finally chosen are in absolute units, but mostly in logarithmic units. The resulting formulations are somewhat more complicated, and, for laboratory workers who may care to use them for current work, we have solved and tabulated the organ weights predicted for each gram of body-weight.

Material.—For the suprarenal we have available the same three series discussed in the paper on heart-weight, and in addition a series of blinded animals and non-blinded controls. Preliminary comparisons showed no material difference between the outdoor daylight subsets within the Compensatory Hypertrophy Series, nor between the Darks and Lights, nor between the Blinded and Non-Blinded. But when the three main series are compared in Fig. 1, we note a striking difference in level between the three regression lines. The Compensatory Hypertrophy Series has the largest SW, next the Dark-Light Series taken as a whole, and smallest the Blinding Experiment. No explanation is apparent.

The magnitude of the difference may be vivified as follows. Take a convenient central body-weight as representative, say 100 g., $\log = 2$, and substitute in the several male equations:

Comp. Hypert. (N 1640):	$Y = .097 + .459\,X = 1.015$,	hence SW =	10.35 mg.
Darks & Lights (N 245):	$Y = -.174 + .585\,X = .996$,	hence SW =	9.91 mg.
Bl. & Non-bl. (N 152):	$Y = -.101 + .538\,X = .975$,	hence SW =	9.46 mg.

Taking the value for the CH series as standard, the DL series was about 4% smaller, and the Blinded-Non-blinded pool was 9% smaller. Therefore pooling would be risky, so we shall take the Compensatory

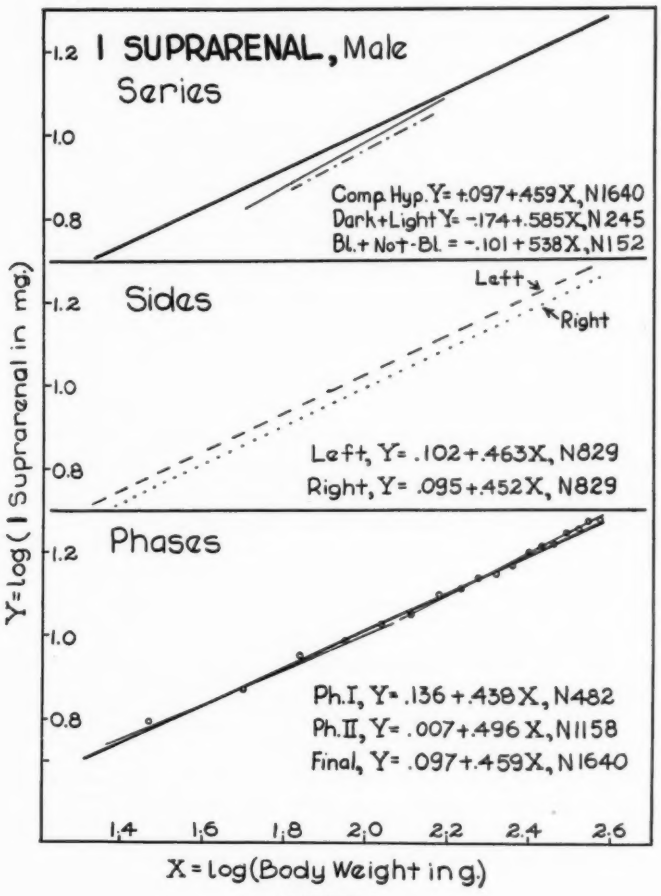

FIGURE 1

Hypertrophy Series, the largest volume of material, as representative, and examine that more particularly.

Basic Constants.—The fundamental reduced data, namely the means and variabilities for suprarenal weight (1 SW) arranged in body weight classes of 20 g. for males, and 10 g. for females, are put on record in Tables 1 and 2.

TABLE 1
SUPRARENAL WEIGHT, 829 MALE ALBINOS
Right and Left Separately

Body Weight in g. 20 g. c. i.	Mid point	No. of Rats f	Suprarenal, Right Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV	Suprarenal, Left Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
20-39	29.5	7	5.86	0.90	0.34	15.4	6.29	0.95	0.36	15.1
40-59	49.5	71	7.15	0.73	0.09	10.2	7.55	0.71	0.08	9.4
60-79	69.5	65	8.58	1.16	0.14	13.5	9.15	1.11	0.14	12.1
80-99	89.5	51	9.63	1.23	0.17	12.8	10.12	1.29	0.18	12.8
100-119	109.5	47	10.26	1.57	0.23	15.3	11.02	1.54	0.22	14.0
120-139	129.5	40	10.82	1.38	0.22	12.7	11.50	1.50	0.24	13.1
140-159	149.5	34	12.03	1.85	0.32	15.4	12.85	1.67	0.29	13.0
160-179	169.5	71	12.80	1.45	0.17	11.3	13.49	1.81	0.22	13.4
180-199	189.5	79	13.32	1.90	0.21	14.3	14.38	1.90	0.21	14.2
200-219	209.5	55	13.65	2.02	0.27	14.8	14.65	2.28	0.31	15.6
220-239	229.5	45	13.98	2.14	0.32	15.3	15.40	2.08	0.31	13.5
240-259	249.5	51	15.14	2.28	0.32	15.1	16.43	2.63	0.37	16.0
260-279	269.5	32	16.00	2.51	0.44	15.7	16.94	2.48	0.44	14.6
280-299	289.5	47	15.91	2.20	0.32	13.8	17.53	2.68	0.39	15.3
300-319	309.5	41	16.85	2.06	0.32	12.2	18.61	2.46	0.38	13.2
320-339	329.5	43	17.23	2.50	0.38	14.5	18.60	2.31	0.35	12.4
340-359	349.5	26	18.00	2.30	0.45	12.8	19.69	2.35	0.46	11.9
360-379	369.5	15	18.47	2.85	0.74	15.4	19.73	2.96	0.77	15.0
380-399	389.5	7	17.71	1.80	0.68	10.2	20.14	3.13	1.18	15.5
400-419	409.5	2	17.50	4.95	3.50	28.3	19.50	7.78	5.50	39.9
		829								

Male Prediction Equations.—The male suprarenal, as noted before, grows like the liver, heart, and kidneys, but unlike the female suprarenal and the gonads in both sexes. As hitherto in this study, we plot in bottom panel of Fig. 1, for each body-weight class log mean BW as X and log mean SW as Y. The points, omitting a few outlying values at the extreme right (380-400 g. and 400-420 g.), which represents respectively only 7 and 2 rats, are well fitted by straight lines. A puberty break is detectible separating two phases, as before. The demarcation appears to lie close to BW 120 g. ($\log = 2.08$), i.e., the same as observed for the heart. If instead of SW by 20-gram BW-classes, we try to locate the point of inflection by plotting SW for each gram of BW, the scatter is so large that it is unintelligible. But on plotting SW representing 5-gram BW-classes, we locate the desired inflection close to 2.07 or 2.08, which confirms the inference stated above. The two phases differ by about the same amount as for heart

TABLE 2
SUPRARENAL WEIGHT, 810 FEMALE ALBINOS
Right and Left Separately

Body Weight in g.			Suprarenal, Right				Suprarenal, Left			
10 g. c. i.	Mid point	No. of Rats f	Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV	Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
40-49	44.5	23	6.67	0.82	0.17	12.3	6.49	1.04	0.22	15.0
50-59	54.5	40	7.50	1.20	0.19	16.0	8.40	1.69	0.27	20.1
60-69	64.5	45	8.77	1.30	0.19	14.8	9.34	1.97	0.29	21.1
70-79	74.5	30	9.50	1.36	0.25	14.4	10.03	1.36	0.25	13.5
80-89	84.5	16	10.88	1.80	0.45	16.6	11.38	2.06	0.52	18.1
90-99	94.5	13	11.73	1.54	0.43	13.1	11.73	1.90	0.53	16.2
100-109	104.5	13	12.19	1.60	0.44	13.1	13.12	1.89	0.52	14.4
110-119	114.5	16	17.00	3.22	0.81	19.0	18.25	3.92	0.98	21.5
120-129	124.5	27	19.61	3.73	0.72	19.0	21.54	3.61	0.70	16.8
130-139	134.5	61	19.42	2.93	0.38	15.1	20.96	3.27	0.42	15.6
140-149	144.5	64	21.41	3.98	0.50	18.6	23.19	3.15	0.39	13.6
150-159	154.5	78	22.22	3.28	0.37	14.8	23.99	3.51	0.40	14.6
160-169	164.5	80	23.80	3.29	0.37	13.8	25.72	3.25	0.36	12.6
170-179	174.5	72	24.42	4.29	0.51	17.6	26.22	4.10	0.48	15.6
180-189	184.5	70	24.19	3.65	0.44	15.1	26.30	3.97	0.48	15.1
190-199	194.5	68	23.56	3.48	0.42	14.8	25.35	3.58	0.43	14.8
200-209	204.5	38	24.24	4.08	0.66	16.9	25.87	3.89	1.16	15.1
210-219	214.5	30	24.37	4.30	0.78	17.6	26.03	3.99	0.73	15.3
220-229	224.5	15	24.50	2.61	0.67	10.6	25.17	2.90	0.75	11.5
230-239	234.5	6	23.17	2.07	0.84	8.9	24.83	2.66	1.08	10.7
240-249	244.5	2	24.50	2.83	2.00	5.8	25.50	4.24	3.00	16.6
250-259	254.5	2	36.50	8.49	6.00	23.2	29.50	7.07	5.00	24.0
260-269	264.5	1	30.50	—	—	—	30.50	—	—	—
		810								

and for kidneys; so that one may wish to use the equation for either the earlier or later phase for maximum precision, but for most purposes a single overall line seems sufficient. Here we give the several equations with Y = log of the average of both suprarenals *as if one organ,* to facilitate comparison on a uniform scale with the equations for right and left suprarenal separately.

Phase I $Y = .136 + .438\ X$, (N 241 rats), n 5
II $Y = .007 + .496\ X$, (N 579 rats), n 13
I and II $Y = .097 + .459\ X$, (N 820 rats), n 18

When it is desired to predict the sum of both SW, one of these equations is solved, converted from logs to milligrams, and then doubled. For the convenience of laboratory work, the equations for the several phases have been solved, doubled and the values of 2 SW predicted for any BW are represented in Table 3 for males.

TABLE 3

STANDARDS FOR 2 SUPRARENALS-WEIGHT IN MALE ALBINO RAT (ADDIS AND GRAY)

Phase I ; for BW 20-119 g.: log (2 SW in mg.) = 0.4378 + 0.4372 log (BW in g.)
Phase II; for BW 120-380 g.: log (2 SW in mg.) = 0.3038 + 0.4980 log (BW in g.)
Eg., for BW = 24 g., predicted 2 SW is 11.0

Body Weight in g.	0	1	2	3	4	5	6	7	8	9
2	10.2	10.4	10.6	10.8	11.0	11.2	11.4	11.6	11.8	12.0
3	12.1	12.3	12.5	12.6	12.8	13.0	13.1	13.3	13.4	13.6
4	13.8	13.9	14.0	14.2	14.3	14.5	14.6	14.8	14.9	15.0
5	15.2	15.3	15.4	15.6	15.7	15.8	15.9	16.0	16.2	16.3
6	16.4	16.5	16.6	16.8	16.9	17.0	17.1	17.2	17.4	17.5
7	17.6	17.7	17.8	17.9	18.0	18.1	18.2	18.3	18.4	18.5
8	18.6	18.7	18.8	18.9	19.0	19.1	19.2	19.3	19.4	19.5
9	19.6	19.7	19.8	19.9	20.0	20.1	20.2	20.3	20.3	20.4
10	20.5	20.6	20.7	20.8	20.9	21.0	21.1	21.1	21.2	21.3
11	21.4	21.5	21.6	21.6	21.7	21.8	21.9	22.0	22.1	22.1
12	21.8	21.9	22.0	22.1	22.2	22.3	22.4	22.5	22.6	22.6
13	22.7	22.8	22.9	23.0	23.1	23.2	23.2	23.3	23.4	23.5
14	23.6	23.7	23.8	23.8	23.9	24.0	24.1	24.2	24.2	24.3
15	24.4	24.5	24.6	24.6	24.7	24.8	24.9	25.0	25.0	25.1
16	25.2	25.3	25.4	25.4	25.5	25.6	25.7	25.8	25.8	25.9
17	26.0	26.0	26.1	26.2	26.3	26.4	26.4	26.5	26.6	26.6
18	26.7	26.8	26.9	27.0	27.0	27.1	27.2	27.2	27.3	27.4
19	27.4	27.5	27.6	27.7	27.7	27.8	27.9	28.0	28.0	28.1
20	28.2	28.2	28.3	28.4	28.4	28.5	28.6	28.7	28.7	28.8
21	28.9	28.9	29.0	29.1	29.1	29.2	29.3	29.3	29.4	29.5
22	29.5	29.6	29.7	29.7	29.8	29.9	30.0	30.0	30.1	30.1
23	30.2	30.3	30.3	30.4	30.5	30.5	30.6	30.7	30.7	30.8
24	30.8	30.9	31.0	31.0	31.1	31.2	31.2	31.3	31.4	31.4
25	31.5	31.5	31.6	31.7	31.7	31.8	31.8	31.9	32.0	32.0
26	32.1	32.2	32.2	32.3	32.3	32.4	32.5	32.5	32.6	32.6
27	32.7	32.8	32.8	32.9	33.0	33.0	33.1	33.1	33.2	33.2
28	33.3	33.4	33.4	33.5	33.5	33.6	33.7	33.7	33.8	33.8
29	33.9	34.0	34.0	34.1	34.1	34.2	34.2	34.3	34.4	34.4
30	34.5	34.5	34.6	34.6	34.7	34.8	34.8	34.9	34.9	35.0
31	35.0	35.1	35.2	35.2	35.3	35.3	35.4	35.4	35.5	35.5
32	35.6	35.6	35.7	35.8	35.8	35.9	35.9	36.0	36.0	36.1
33	36.1	36.2	36.3	36.3	36.4	36.4	36.5	36.5	36.6	36.6
34	36.7	36.7	36.8	36.8	36.9	37.0	37.0	37.1	37.1	37.2
35	37.2	37.3	37.3	37.4	37.4	37.5	37.5	37.6	37.6	37.7
36	37.7	37.8	37.8	37.9	38.0	38.0	38.1	38.1	38.2	38.2
37	38.3	38.3	38.4	38.4	38.5	38.5	38.6	38.6	38.7	38.7
38	38.8									

Female Prediction Equation.—Unlike heart and kidney, and male suprarenal, the female suprarenal grows in a way markedly different.

For females the material used was the asymmetry series plus the non-blinded series. No straight-line function fits Fig. 2. The two phases meet at a cusp, which seems to lie close to 2.02, which is the logarithm of 105 g. BW. Closer examination by the same method as with males, namely plotting SW means for each gram BW, gives a graph with a cusp close to 2.025, i.e., log 106 grams BW, which confirms the prior estimate. And again, plotting values for BW-classes of 5 grams each (not reproduced) the same estimate is evident.

The several equations are given for the sum of both suprarenals. Here in the case of the peculiar female curve, the labor of computation is so heavy that the left and right organs are not shown separately. The form of equation used is not pretended to be by any means ideal, but it does seem to smooth the observed data nicely enough for practical purposes.

The equations fitted are:

$$\text{Phase I} \qquad Y = 0.538 + 2.769 \log X,$$
$$\log (2 \text{ SW in mg.}) = 0.538 + 2.769 \log \log (\text{BW in g.}),$$

with error —2.3 to +1.7, averaging 1.3%; range of BW 40-105 g.

$$\text{Phase II} \qquad Y = 1.6604 - .019125z - .0027084z^2 - .0001\ 1281z^3,$$
$$\text{where} \qquad Y = \log (2 \text{ SW in mg.})$$
$$z = \left[\frac{1}{\log (\text{BW in g.})} - .4610 \right] / .005,$$
$$\text{Range} = \text{BW } 105\text{-}250 \text{ g.};$$

with error —8.2 to +4.0, averaging 1.6%. For the convenience of laboratory work, the equations for the several phases have been solved, and the values of 2 SW predicted for any BW are represented in Table 4 for females.

Prediction Errors.—For the predicted weight in milligrams from male overall equation, the percentage prediction errors for the 18 rows or body-weight groups ran from +4.3 to —7.7%, averaging 2.0%. For the females, phase I, the error varied from 1.7 to —2.3, averaging 1.3%; and phase II, from +4.0 to —8.2, averaging 1.6%. These were considered eminently satisfactory.

Variability.—Some quantitative conception of the biological fluctuation in organ weight within a group of animals. Using age as the basis of grouping, Jackson found coefficients of variation in SW which ranged from 20 to 39%, with an average of 26%. This is of the same order as he found for heart and kidneys. But, as we must emphasize,

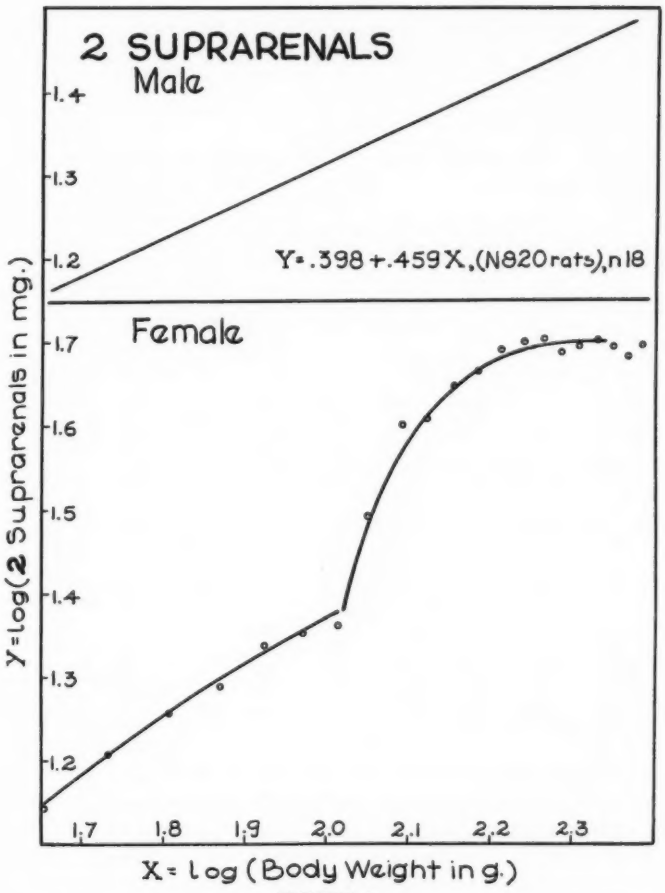

FIGURE 2

Both panels have the same scale for visualization of the extraordinary difference in manner of growth in the two sexes.

Upper panel: the male line for the sum of 2 suprarenals represents the equation

$$\log (2\ SW) = .398 + .459\ X$$

which is derived directly from the final equation shown in Fig. 2.

Lower panel: the female curves are given in the text. It will be noticed that in both phases repeated transformations were used in order to obtain suitable and convenient working units for body-weight. The working unit was not X or log BW as in the equations for liver, heart, kidney and male suprarenal. For phase I the working unit was log X or log log BW, and for phase II z was a more complicated function of body-weight.

TABLE 4

STANDARDS FOR 2 SUPRARENALS-WEIGHT IN FEMALE ALBINO RAT (ADDIS AND GRAY)

Phase I for BW 40-105 g.: log (2 SW in mg.) = 0.538 + 2.769 log log (BW in g.)

Phase II for BW 106-250 g.: log (2 SW in mg.) = $1.6604 - .019125z - .0027084z^2$ $-.0001\ 1281z^3$ and where $z = \left[\frac{1}{\log (\text{BW in g.})} - .4610 \right] / .005$

Eg., for BW = 44 g., predicted 2 SW is 13.7 mg.

Body Weight in g.	0	1	2	3	4	5	6	7	8	9
4	12.7	13.0	13.2	13.4	13.7	13.9	14.1	14.3	14.6	14.8
5	15.0	15.2	15.4	15.6	15.8	16.0	16.2	16.4	16.6	16.8
6	17.0	17.2	17.4	17.6	17.8	17.9	18.1	18.3	18.5	18.7
7	18.8	19.0	19.2	19.4	19.5	19.7	19.9	20.0	20.2	20.3
8	20.5	20.7	20.8	21.0	21.2	21.3	21.5	21.6	21.8	21.9
9	22.1	22.2	22.4	22.5	22.7	22.8	23.0	23.1	23.2	23.4
10	23.5	23.7	23.8	23.9	24.1	24.2	24.3	25.1	25.8	26.6
11	27.3	28.1	28.8	29.6	30.3	31.0	31.6	32.2	33.0	33.7
12	34.1	34.8	35.3	36.0	36.4	37.1	37.5	38.1	38.5	39.0
13	39.5	39.9	40.5	40.9	41.3	41.7	42.2	42.5	42.9	43.2
14	43.5	43.9	44.2	44.5	44.8	45.1	45.3	45.5	45.8	46.0
15	46.3	46.6	46.7	47.0	47.2	47.3	47.5	47.8	47.9	48.0
16	48.2	48.3	48.5	48.5	48.8	48.9	49.0	49.1	49.2	49.3
17	49.3	49.4	49.5	49.7	49.7	49.8	49.8	49.9	49.9	50.0
18	50.0	50.1	50.1	50.1	50.1	50.2	50.2	50.2	50.2	50.4
19	50.4	50.4	50.4	50.4	50.4	50.4	50.4	50.4	50.4	50.4
20	50.4	50.4	50.4	50.4	50.4	50.4	50.4	50.4	50.4	50.4
21	50.4	50.4	50.4	50.4	50.2	50.2	50.2	50.2	50.2	50.2
22	50.2	50.1	50.1	50.1	50.1	50.1	50.1	50.1	50.0	50.0
23	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
24	50.0	49.8	49.8	49.8	49.8	49.8	49.7	49.7	49.7	49.7

this is too fluctuant to be of any use. Some other base of grouping is needed, such as body weight. Our estimate, by grouping the rats in body-weight classes, yielded coefficients of variation averaging 8% for heart weight, 9% for kidney weight. By the same method we now find for SW the CV averages about 15%. The reduced details are shown in the accompanying text-table.

Asymmetry.—The rat's right suprarenal is smaller than the left, by about 7%. This is the reverse of the findings for the kidneys. This quantitative comparison was arrived at by the procedure described in the paper on asymmetry of the kidneys. The resultant right/left ratio affords a measure of bilateral asymmetry. It was found that the difference between the sex means, referred to the standard error of

Sex	Side	Groups: No. of Rats	Groups: BW-classes	Groups: Range	Coefficient of Variation: Mean CV	Coefficient of Variation: Standard Deviation	Coefficient of Variation: Standard Error of Mean
Male	R	822	17	10.2-15.7	13.8	2.61	.392
	L	822	17	9.4-16.0	13.5	2.60	.391
Female	R	799	19	12.3-19.0	15.4	2.33	.535
	L	799	19	11.5-21.5	15.8	2.70	.618

that difference, was statistically non-significant. Details have been reduced to the attached text-table.

Sex	Rats N	Classes f	Mean M	Standard Deviation	Standard Error of Mean
Male	829	17	.930	.015	.004
Female	810	19	.937	.024	.005

Graphically, for males, the larger left and the smaller right suprarenal can be seen in Fig. 1, above and below the panel representing the average of the two sides. The respective equations, all in terms of 1 SW, are:

Joint RT Lt,	$Y = .097 + .459\ X$, N	1640 organs
Left	$Y = .102 + .463\ X$, N	820 organs
Right	$Y = .095 + .452\ X$, N	820 organs

For females the equations are so complex that they were not computed separately for left and right.

Comparison with Other Series.—Hatai's males, whether expressed in the line given by Brody or as computed by us, log (1 SW in mg.) = —.254 + .647 log (BW in grams), have a steeper rate of relative growth, a larger percentage prediction error, around 10% and cross the line for our material at about 100 grams body-weight. Hence no satisfactory comparison can be made.

Laqueur's 115 females if plotted would be distributed fairly well around our line, and therefore his material and ours may be said to be in conformity for the suprarenals, unlike what we pointed out elsewhere for kidney weight, where for his animals the 2 KW for set body-weights ran about 28% above ours.

Summary.—This is the second of three contributions of predicted values for organ-weight in albino rats under exceptionally uniform conditions. The first paper dealt with the heart, the kidneys, and the

liver, all in units of logarithms of body-weight and organ weight, i.e., simplification by rectification of the parabolic equations to straight lines. The present paper treats the suprarenals. The third and final paper will show gonad weights, well fitted by a still more complex formula. For all the material, the recommended equations have been solved at frequent suitable intervals of body-weight, to furnish the experimenter with convenient tables of prediction.

References

Addis, T., & Gray, H. 1950. Body Weight and Organ Weight. In press.

Donaldson, H. H. 1924. The Rat, Wistar Institute, Philadelphia, edition 2, pp. xiv+469.

Lawrence, J. H., Hamilton, J. G., Erf, L. A., & Pecher, C. 1941. Recent Advances in Clinical Medicine with the Aid of Artificially Prepared Radioactive Isotopes. *J. Clin. Invest.*, **20**, 436.

Levine, C. J., Mann, W., Hodge, H. C., Ariel, I., & DuPont, O. 1941. Distribution of Body Weight in the Organs and Tissues of the Rabbit. *Proc. Soc. Exp. Biol. and Med.*, **47**, 318-321.

Ross, J. F., & Chapin, M. A. 1941. The Selective Absorption of Radioactive Iron. *J. Clin. Invest.*, **20**, 437.

Slonaker, J. R., & Card, T. A. 1923. The Effect of a Restricted Diet on Growth. *Am. J. of Phys.*, **63**, 503-512.

The other references have been cited already in Paper I, namely Brody, Hatai, Jackson, Laqueur, Walter and Addis.

Growth, 1950, **14**, 93-106.

BODY SIZE AND GONAD WEIGHT

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INTRODUCTION

Two preceding papers have urged the utility of standards obtained from rats kept under carefully controlled conditions; the data smoothed and fitted by equations, to facilitate prediction of organ-weight in experimental work, whenever such standards are suitable enough to dispense with the labor and cost of running parallel controls. The present paper is the third and final part of the work, dealing with the gonads, which have required a particularly laborious equation. But, as in the previous papers, the equations have been solved at convenient intervals, to afford handy tables for prediction.

GONAD-WEIGHT (GW)

Material.—We have the same series as in the preceding section on suprarenal-weight. Preliminary comparisons between the outdoor sunlight subsets within the Compensatory Hypertrophy Series, showed nothing for females, but for males gave some support to the sequence found for liver-weight. That is, the longer the outdoor "sunlight", the lower the gonad-weight; and here again the unexplained exception that the subset with the longest exposure had larger gonads than the other three subsets, not smaller as expected. But since the order of the differences and also the degrees of difference were inconclusive, the Compensatory Hypertrophy Series will here, as before, be treated as a whole.

The Lights plotted above the Darks, for each sex. Here is something significant, which was not in evidence for any other organs than the testicles and ovaries. Quantitative assessment of the degree of difference is shown in Table 1. From these the index D/L is seen to vary in the ten groups from 0.66 to 1.23, averaging 0.87, i.e., the

TABLE 1
GONADS IN THE DARK VS. THE LIGHT

Body Weight Class	mp	Weight 2 Gonads (Sum Both Sides) 245 Males f D	L	Mean D	L	Index D/L	268 Females f D	L	Mean D	L	Index D/L
40-49	44.5	..	..	..	..	..	0	1	..	16.5	..
50-59	54.5	4	4	474	374	1.27	2	2	17.5	18.5	.95
60-69	64.5	4	4	500	550	.91	5	8	20.5	22.2	.92
70-79	74.5	15	6	523	716	.73	14	17	22.6	28.9	.78
80-89	84.5	12	11	500	759	.66	25	37	28.4	31.6	.90
90-99	94.5	30	28	740	935	.79	33	55	35.6	40.2	.89
100-109	104.5	18	31	844	1079	.78	24	19	41.3	45.1	.92
110-119	114.5	23	17	1067	1408	.76	13	9	50.0	51.4	.97
120-129	124.5	12	10	1466	1559	.94	2	0	47.5	..	..
130-139	134.5	7	2	1621	1749	.93	1	0	58.5	..	..
140-149	144.5	3	0	1683	..	..	1	0	66.5	..	..
150-159	154.5	3	1	1916	2050	.94	.	.	..	..	..
		131	114			.87	120	148			.90

Darks were 13% smaller than the Lights. Correspondingly, the D/L index in seven body-weight groups of females ran from 0.78 to 0.97, averaging 0.90, i.e., the Darks 10% smaller than the Lights.

Blinding.—The blinded subseries showed smaller gonads than the non-blinded controls, in both sexes. Again we have a discovery not found for other organs. The diagrams are omitted but quantitative comparison of the degree of effect of this experimental factor may be recorded in Table 2. From these the index B/NB is seen for 7 male

TABLE 2
GONADS IN BLINDED VS. NON-BLINDED

Body Weight Class	mp	Weight 2 Gonads (Sum Both Sides) 158 Males f B	NB	Mean B	NB	Index B/NB	146 Females f B	NB	Mean B	NB	Index B/NB
60-69	64.5	1	0	850		.	0	0		..	..
70-79	74.5	0	1	..	750	..	4	0	27.5	..	..
80-89	84.5	4	0	974			12	3	27.8	25.8	1.08
90-99	94.5	8	5	812	1010	.80	24	11	30.2	30.3	1.00
100-109	104.5	13	9	911	1105	.82	35	19	35.0	36.9	.95
110-119	114.5	22	17	990	1432	.69	19	11	40.7	46.9	.87
120-129	124.5	14	11	1371	1377	1.00	6	1	47.2	50.5	.94
130-139	134.5	20	18	1565	1727	.91	1	0	56.5	..	..
140-149	144.5	9	2	1727	1550	1.11					
150-159	154.5	1	2	1750	1800	.97					
160-169	164.5	0	1	..	1950	.					
		92	66			.90	101	45			.97

groups to vary from 0.69 to 1.11, average 0.90%, i.e, the testes in Blinded were 10% smaller than in the controls. Correspondingly, five female groups had the index from 0.86 to 1.08, average 0.96, i.e., ovaries in the Blinded were 4% smaller than in the Non-Blinded controls.

Three Main Series Compared.—The Darks and Lights together or the Blinded Series, differ from the Compensatory Hypertrophy Series sufficiently, but also so irregularly as to make graphing unsatisfactory. The last named series will therefore be used to obtain standard averages in what follows immediately.

Basic Constants.—The fundamental reduced data are reported for the means and variabilities for gonad weight, arranged by body-weight classes of 20 grams for males and 10 grams for females and are made a matter of record. Owing to the marked bilateral asymmetry, the right and left gonads are listed separately in Tables 3 and 4.

TABLE 3
GONAD WEIGHT, 829 MALE ALBINOS
Right and Left Separately

Body Weight in g. 20 g. c. i.	mp	No. of Rats f	Gonad, Right Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV	Gonad, Left Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
20-39	29.5	7	92	53	20	57.9	107	53	20	50.1
40-59	49.5	71	166	77	9	46.5	172	73	9	42.6
60-79	69.5	65	226	100	12	44.0	239	100	12	42.0
80-99	89.5	51	369	139	19	37.5	379	145	20	38.2
100-119	109.5	47	494	182	26	36.7	520	206	30	39.7
120-139	129.5	40	660	193	31	29.3	692	211	33	30.5
140-159	149.5	34	847	236	40	27.8	864	235	40	27.2
160-179	169.5	71	1019	253	30	24.8	1047	265	32	25.3
180-199	189.5	79	1125	240	27	21.3	1153	240	27	20.8
200-219	209.5	55	1119	266	36	23.8	1144	264	36	23.1
220-239	229.5	45	1161	224	33	19.3	1198	218	33	18.2
240-259	249.5	51	1214	214	30	17.6	1257	224	31	17.8
260-279	269.5	32	1278	183	32	14.3	1290	185	33	14.3
280-299	289.5	47	1239	211	31	17.0	1275	214	31	16.8
300-319	309.5	41	1425	141	22	9.9	1442	178	28	12.4
320-339	329.5	43	1389	185	28	13.3	1424	197	30	13.8
340-359	349.5	26	1407	212	42	15.1	1450	215	42	14.9
360-379	369.5	15	1590	206	53	13.0	1550	136	35	8.8
380-399	389.5	7	1464	147	55	10.0	1550	129	49	8.3
400-419	409.5	2	1500	71	50	4.7	1600	71	50	4.4
		829								

TABLE 4
GONAD WEIGHT, 810 FEMALE ALBINOS
Right and Left Separately

Body Weight in g. 10 g. c. i.	mp	No. of Rats f	Gonad, Right Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV	Gonad, Left Mean M	Standard Deviation SD	Standard Error of Mean SE	Coef. of Variability CV
40-49	44.5	23	9.8	2.53	0.53	25.8	10.0	2.50	0.52	24.8
50-59	54.5	40	11.9	1.79	0.28	15.0	12.0	3.49	0.55	29.1
60-69	64.5	45	12.8	2.55	0.38	20.0	12.9	2.16	0.32	16.7
70-79	74.5	30	13.2	2.84	0.52	21.6	13.7	2.40	0.44	17.5
80-89	84.5	16	14.8	3.15	0.79	21.2	16.7	2.21	0.55	13.3
90-99	94.5	13	17.0	2.89	0.80	17.0	16.2	3.44	0.96	21.2
100-109	104.5	13	18.5	4.74	1.31	25.5	19.7	7.27	2.02	36.9
110-119	114.5	16	26.1	8.80	2.20	33.7	27.9	8.01	2.00	28.7
120-129	124.5	27	35.5	7.82	1.51	22.0	34.4	6.26	1.21	18.2
130-139	134.5	61	36.8	6.13	0.78	16.6	37.5	8.33	1.07	22.2
140-149	144.5	64	39.3	8.12	1.01	20.7	39.0	8.29	1.04	21.3
150-159	154.5	78	41.4	2.85	0.32	21.3	41.7	7.87	0.89	18.9
160-169	164.5	80	42.8	6.57	0.73	15.4	42.7	7.50	0.84	17.6
170-179	174.5	72	44.0	5.86	0.69	13.3	45.4	6.16	0.73	13.6
180-189	184.5	70	45.4	7.25	0.87	16.0	45.8	7.10	0.85	15.5
190-199	194.5	68	44.6	7.61	0.92	17.1	44.9	7.35	0.89	16.3
200-209	204.5	38	44.4	7.95	1.29	17.9	44.6	7.95	1.29	17.8
210-219	214.5	30	40.5	5.60	1.02	13.8	42.7	6.67	1.22	15.5
220-229	224.5	15	42.3	4.42	1.14	10.4	44.0	7.98	2.06	18.1
230-239	234.5	6	45.3	7.53	3.01	16.6	42.0	4.47	1.79	10.6
240-249	244.5	2	44.5	—	—	—	44.5	—	—	—
250-259	254.5	2	47.0	—	—	—	49.5	—	—	—
260-269	264.5	1	37.0	—	—	—	57.0	—	—	—
		810								

Male Prediction Equations.—The male gonad, as noted before, grows somewhat like the liver, heart, and kidneys, but quite unlike the female suprarenals and the gonads. As hitherto in this study, we plot in Fig. 1, for each body-weight class the mean BW as X and the mean GW as Y. The points are not well fitted by straight lines. If we plot absolute values in Fig. 2, upper panel, the means are now fitted by straight lines, in three phases.

Phases of growth for this organ, the male testicle, are seen to be not two as for other organs but three. The early break is detectible at the midpoint of the BW class 60-79 g., i.e., at 69.5 grams or 1.842 on the log-scale. The second or puberty break is seen somewhere in the range of the BW classes 160-180-200 grams, say at 180 grams, or 2.255 on the log-scale. This point at 180 g. is notably later than that

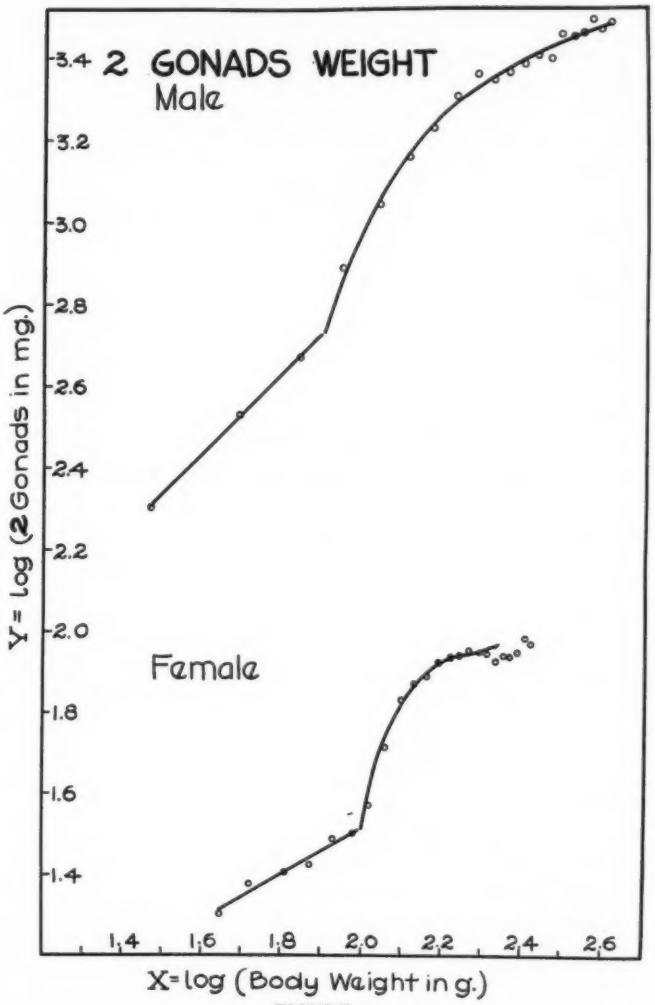

FIGURE 1

pointed out for the kidney which was at 100 grams BW or log = 2.00; and also later than that pointed out for the heart which was close to 120 grams or log = 2.08. These distinctions may be important.

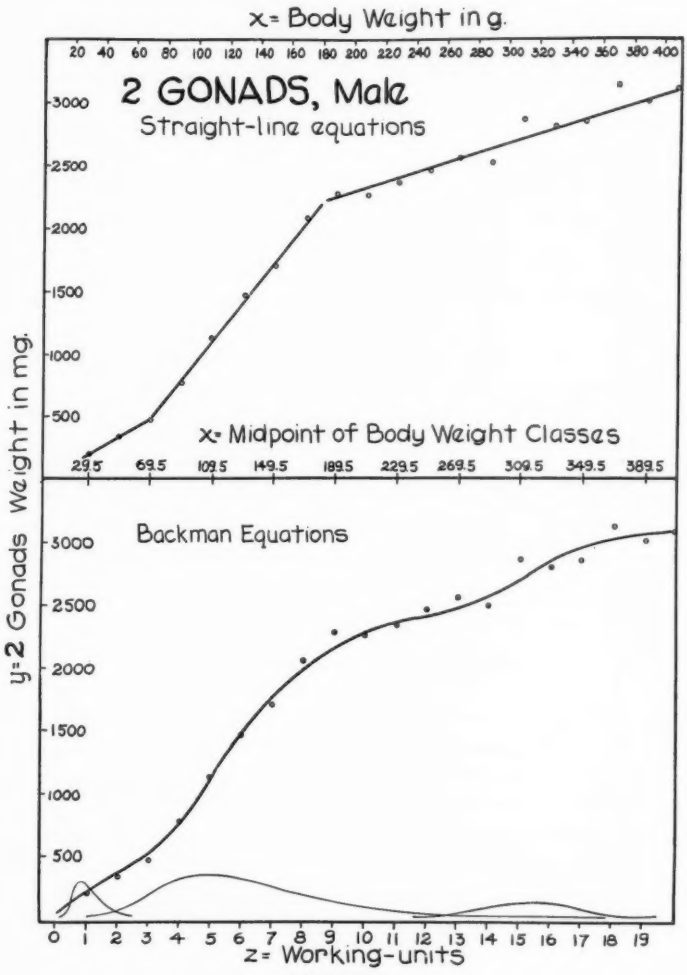

FIGURE 2

If instead of GW by 20-gram BW-classes, we try to locate the point of inflection by plotting GW for each gram of BW, the scatter is so large that it is unintelligible. But on plotting GW representing 5-gram

BW-classes, we locate the desired inflection close to 2.11 or about 129 grams which suggests a somewhat earlier location than that inferred in the preceding paragraph. The different result when using logarithmic working units is puzzling. At all events, we may say that even then the earliest demarcating point for the break at 2.11 confirms the results reached in the prior paragraph that the puberty break for gonads is later than for heart and kidneys.

In sum, the breaks between the phases are so much more marked in degree than for the heart and for kidneys that one should use a different equation according to phase for maximum precision. For no purposes is a single overall line sufficient.

Three Straight Lines.—We have fitted the plotted means by two kinds of formula, by ordinary straight lines and by Backman's function. For the straight lines we give the several equations $y = b + kx$, where x = midpoint of BW-class in grams (N.B., not logs), y = the average in mg. of both gonads as if one organ, to facilitate comparison on a uniform scale with the lines for right and left separately.

Phase I 1 GW = —.05 + 3.386 BW, (N 144 males) n 3, for BW 20-70 g.
Phase II = —315.2 + 7.926 BW, (N 374 M) n 6, for BW 70-180 g.
Phase III = +740.3 + 1.982 BW, (N 443 M) n 12, for BW 180-420 g.

When it is desired to predict the sum of both GW, the equation above appropriate to given body-weight is solved and then doubled; or one may transform the equations and solve as presented for convenient use in Table 5.

The male means got from Table 3 are plotted in Fig. 2, upper panel, together with these three phase lines. The prediction error for these three equations ranged from —6.2 to +4.5 averaging without regard to sign 1.64%, with standard deviation = 1.78 and standard error of the mean (average) = 0.39.

Backman Functions.—This procedure is based on the hypotheses that the total growth curve can often be best represented by treatment in several phases or cycles, that these are best treated primarily in terms of rates or Functions, F, and only secondly in absolute magnitudes, and that the underlying growth impulses in the several cycles overlap to some extent. We have

General F $= k_0 + k_1 \log x + k_2 (\log x)^2$

Phase I: F_I = antilog of (—.5472 —.8262 log x —5.1994 $\log^2 x$), and in this column mark the values at $x = 0.5, 1.5, 2.5, 3.5$, which are the appropriate items to sum later with F_{II}. Also since x is used at intervals $\Delta x = 0.1$, y theoretical = 0.1 F_I.

TABLE 5

STANDARDS FOR 2 GONADS-WEIGHT IN MALE ALBINO RAT (ADDIS AND GRAY)

Phase I ; for BW 20-69 g.: (2 GW in mg.) = 0.2 + 6.766 (BW in g.)
Phase II ; for BW 70-177 g.: (2 GW in mg.) = —630.4 + 15.852 (BW in g.)
Phase III; for BW 178-420 g.: (2 GW in mg.) = 1482.6 + 3.953 (BW in g.)

Eg., for BW = 24 g., predicted 2 GW is 163 mg.

Body Weight in g.	0	1	2	3	4	5	6	7	8	9
2	136	142	149	156	163	169	176	183	190	196
3	203	210	217	224	230	237	244	250	257	264
4	271	278	286	291	298	305	311	318	325	332
5	338	345	352	359	366	372	379	386	393	399
6	406	413	420	426	433	440	447	454	460	467
7	479	495	511	527	543	558	574	590	606	623
8	638	654	669	685	701	717	733	749	764	780
9	796	812	828	844	860	876	891	907	923	939
10	955	971	986	1002	1018	1034	1050	1066	1082	1097
11	1113	1129	1145	1161	1177	1192	1208	1224	1240	1256
12	1272	1288	1304	1319	1335	1351	1367	1383	1399	1414
13	1430	1446	1462	1478	1494	1510	1525	1541	1557	1573
14	1589	1605	1620	1636	1652	1668	1684	1700	1716	1732
15	1747	1763	1779	1795	1811	1827	1842	1858	1874	1890
16	1906	1922	1938	1953	1969	1985	2001	2017	2033	2048
17	2064	2080	2096	2112	2128	2144	2160	2175	2186	2190
18	2194	2198	2202	2206	2210	2214	2218	2222	2226	2230
19	2234	2238	2242	2246	2250	2254	2257	2261	2265	2269
20	2273	2277	2281	2285	2289	2293	2297	2301	2305	2309
21	2313	2317	2321	2325	2328	2332	2336	2340	2344	2348
22	2352	2356	2360	2364	2368	2372	2376	2380	2384	2388
23	2392	2396	2400	2404	2408	2412	2416	2419	2423	2427
24	2431	2435	2439	2443	2447	2451	2455	2459	2463	2467
25	2471	2475	2479	2483	2487	2491	2495	2498	2502	2506
26	2510	2514	2518	2522	2526	2530	2534	2538	2542	2546
27	2550	2554	2558	2562	2566	2570	2574	2578	2582	2586
28	2589	2593	2597	2601	2605	2609	2613	2617	2621	2625
29	2629	2633	2637	2641	2645	2649	2653	2657	2661	2664
30	2668	2672	2676	2680	2684	2688	2692	2696	2700	2704
31	2708	2712	2716	2720	2724	2728	2732	2736	2740	2744
32	2748	2752	2755	2759	2763	2767	2771	2775	2779	2783
33	2787	2791	2795	2799	2803	2807	2811	2815	2819	2823
34	2827	2831	2834	2838	2842	2846	2850	2854	2858	2862
35	2866	2870	2874	2878	2882	2886	2890	2894	2898	2902
36	2906	2910	2914	2918	2922	2925	2929	2933	2937	2941
37	2945	2949	2953	2957	2961	2965	2969	2973	2977	2981
38	2985	2989	2993	2997	3000	3004	3008	3012	3016	3020
39	3024	3028	3032	3036	3040	3044	3048	3052	3056	3060
40	3064	3068	3072	3076	3080	3084	3088	3091	3095	3099
41	3103	3107	3111	3115	3119	3123	3127	3131	3135	3139

Phase II: F_{II} = antilog of $(-3.2737 + 8.1590 \log x - 5.8840 (\log x)^2)$
y at x = 0, i.e., 9.5, midpoint 0-19 g. BW, assumed = .03
y at x = 1, i.e., 29.5, midpoint 20-39 g. = 0.3 + F_I for x = 0.5 namely .17, plus F_{II} for x = 0.5, namely 0; total .20.
y at x = 2, i.e., 49.5, midpoint 40-59 g. = last named total (.20) plus F_I for x = 1.5, namely .14, plus F_{II} for x = 1.5 namely .01, giving total .35, and so forth.

Phase III: F_{III} = antilog of $(-146.33 + 245.07 \log x - 103.22 \log 2x)$
This column of values for x 11.5 reads 0.00, for 12.5 reads .02 and so forth till for x 20.5 it decreased again to 0.00.

These three phase values have to be summed with the sum of columns F_I and F_{II}, giving the final y's predicted, stated in working-units of grams. The resulting curves, both for the rate $\Delta y/\Delta x$ and for the summated curve of GW is shown in Fig. 2, lower panel.

Prediction Error.—Since the computations were done for y = 2 GW in gram-units, multiply by 1000 to convert to milligram units and then compare with original GW, 194.0, 337.0 and so forth. For 20 points the percentage error ranged from —5.9 to +3.9 averaging without regard to sign 2.48%, with standard deviation = 1.71, and standard error of the average = 0.38.

Concluding Choice.—Both forms of equation yield excellent fits to the means observed. The Backman equations have the advantage of a theoretical rationale for growth and a smaller variation in percentage error. The straight-line equations have the advantage of a smaller average error and of much greater ease of computation.

Female Prediction Equations.—The female gonad grows much the same as the female suprarenal. As found convenient in this study for other organs except the testicle, we have converted the averages into logarithmic working units, log BW in grams = X, log of Gonad Weight in mg. = Y, and have so plotted in Fig. 1. Four points at the right (for BW classes centered at 214.5, 224.5, 234.5, 244.5) indicate some peculiarity in the observed data, most likely a physiological but nevertheless peculiar obesity. Ignoring these four values, we find two phases meeting at a cusp. It seems to lie close to 2.00, i.e., log 100 g. BW. Closer examination by the same method used for SW, namely with values of GW for smaller BW-classes of 5 grams (instead of the usual 10), puts inflection at about 2.01 to 2.03, resembling the female suprarenals. But on the whole the time of this puberty break confirms satisfactorily that noted for the female heart and suprarenals.

The equations fitted to the sum of the 2 GW are:

Phase I, for the range 40 to 105 g. BW, $Y = .7878 + 2.4102\ U$
where $U = \log X = \log \log$ BW in g.

The solved values are converted from logs into absolute units, the deviations of those predictions from the original observed means for the several BW classes, are noted, then expressed as a percentage above or below the observed. These percentage prediction errors ranged from +3.5 to —2.9 and averaged 2.21%.

TABLE 6

STANDARDS FOR 2 GONADS-WEIGHT IN FEMALE ALBINO RAT (ADDIS AND GRAY)

Phase I for BW 40-105 g.: log (2 GW in mg.) = .7878 + 2.4102 U, where U = log X = log log (BW in g.)

Phase II for BW 106-205 g.: log (2 GW in mg.) = $1.9104 - .016586z - .022\ 6415z^2 - .0002\ 3054z^3$, and where $z = \left[\frac{1}{\log (\text{BW in g.})} - .4610\right] / .005$

Eg., for BW = 44 g., predicted 2 GW is 20.3 mg.

	0	1	2	3	4	5	6	7	8	9
4	19.1	19.4	19.7	20.0	20.3	20.6	20.9	21.2	21.5	21.7
5	22.0	22.3	22.5	22.8	23.1	23.3	23.6	23.8	24.1	24.3
6	24.6	24.8	25.0	25.3	25.5	25.7	26.0	26.2	26.4	26.6
7	26.8	27.1	27.3	27.5	27.7	27.9	28.1	28.3	28.5	28.7
8	28.9	29.1	29.3	29.5	29.7	29.9	30.1	30.3	30.5	30.7
9	30.8	31.0	31.2	31.4	31.6	31.7	31.9	32.1	32.3	32.4
10	32.6	32.8	32.9	33.1	33.3	33.5	42.0	43.4	44.9	46.5
11	47.9	49.5	51.0	52.4	53.9	55.2	56.2	57.6	59.0	60.3
12	61.2	62.5	63.4	64.6	65.5	66.6	67.5	68.5	69.4	70.1
13	71.2	71.8	72.8	73.5	74.1	74.7	75.6	76.2	76.7	77.3
14	77.8	78.3	78.9	79.3	79.8	80.3	80.7	81.1	81.5	81.8
15	82.2	82.6	82.8	83.2	83.6	83.8	84.1	84.3	84.5	84.7
16	85.1	85.3	85.5	85.7	85.9	86.1	86.3	86.5	86.7	86.7
17	86.9	87.1	87.3	87.5	87.5	87.7	87.9	87.9	88.1	88.1
18	88.3	88.5	88.5	88.7	88.9	88.9	89.1	89.1	89.3	89.3
19	89.5	89.7	89.7	89.9	89.9	90.2	90.4	90.4	90.6	90.8
20	90.8	91.0	91.2	91.2	91.4	91.6				

Phase II, range 105 g. to 205 g. (i.e., shorter than for SW) gives the cubic parabola:

$$Y = 1.9104 - .016586z - .0026415z^2 - .00023054z^3$$

where $Y = \log$ (2 GW in mg.)

$$z = \left[\frac{1}{\log \text{BW in g.}} - .4610\right] / .005,$$

i.e., the same working units as in the female suprarenal equation.

Then the errors ran from +6.6 to —2.5, averaging 2.45%. These fits are satisfactory.

Sex Difference.—For any given body-weight, the male gonads are twenty-two times as heavy as the female. This figure was derived by summing the two sides from Table 3 and 4, and furthermore for the females, by averaging the two sums for successive 10-gram BW-classes, two at a time, to obtain averages comparable with those for male 20-gram classes. Then, for each class, the male average was divided by the female. There were 11 comparable averages, yielding indices ranging from 15.5 to 27.2, and averaging 22. This device manifests the phenomenal sex-difference much more plainly than do the graphs which demand such divergent scale-units.

Variability.—In the gonads biological variation is particularly large. For example, for 2 testes weight in albino rats at 6 set ages: Birth, 7 days, 21, 41, 70, and 150 days, Jackson reported the coefficient of variation as ranging from 18 to 41%, with an average of 29%, and for ovaries at the last 5 ages, from 33 to 51%, with an average of 43%.

And for a contrasting example, in this colony, grouped by body-weight in 20 gram class-intervals, omitting the classes with frequencies of 10 or fewer, the results are given in the adjoining text-table. In addition to the fact that variability in gonad weight is much greater than in the other organs here reported, a minor point may be noted, namely that the coefficient itself is extraordinarily labile or inconstant among the several BW-classes, as indicated by the high standard deviation for females, and still more so for males.

Sex	Side	No. of rats	BW-classes	Mean	Standard Deviation	Standard Error of Mean
Male	R	813	17	24.2	11.2	2.72
	L	813	17	23.9	11.1	2.68
Female	R	799	19	19.2	5.4	1.23
	L	799	19	20.2	6.0	1.38

Asymmetry.—Unlike the kidneys, but like the suprarenals, the gonads are smaller on the right side than the left, for this organ by about 3%. This quantitative comparison was arrived at by the following procedure: a table was drawn up listing our usual body-weight classes, the mean right GW, the mean left GW, and the ratio between

the two sides. This may be called the asymmetry or R/L index. Its values may be handled in conventional manner; the classes with frequencies, f, of 10 or fewer rats have been omitted because such small numbers yielded such wide fluctuations of the index (3 male classes and 4 females); and for each remaining class the frequency less one, f—1, was used as Fisher's degrees of freedom in computing the formulas. The resulting constants are shown in the accompanying text-table. The difference between the sex means in relation to the standard error of that difference, was statistically non-significant.

Sex	Rats N	Classes f	Mean M	Standard Deviation	Standard Error of Mean
Male	813	17	.974	.018	.004
Female	799	19	.979	.036	.008

Comparison with Other Series.—Hatai's males had heavier testes at body-weights under about 170 grams, and thereafter conform fairly closely to ours (Fig. 3). His females lie far below ours. The difference is due in part to the fact that here the technique was to remove the ovaries with the capsule. But there must be a further factor, because, even when stripped down to the parenchyma, ovaries from other series behave differently again. Two examples are shown in Fig. 3. The averages from Laqueur and Fluhmann (1942), each solid square representing an average from 3 to 5 rats, show the same marked increase in gonad weight for the period during which BW increased from about 130 to 180 g. And Laqueur's unpublished means on 115 rats show 9 means which on the whole are parallel below our line and above Hatai's; yet do show one very high average 90 grams GW for 4 rats in the BW-class 220-229 grams.

In retrospect, it cannot be determined how far these divergences may be due to menstrual cycles, which were not recorded in general. Dr. Laqueur tells us that his material, when so subdivided, shows that the ovaries were heavier by a statistically significant amount for the 7 rats killed during metestrus, with no significant distinctions in ovary weight between the 22 rats in proestrus, the 38 in estrus, or the 44 in diestrus. A like regard for the cycle in future seems indicated for the female suprarenals, which were heavier during another cycle, to wit, estrus.

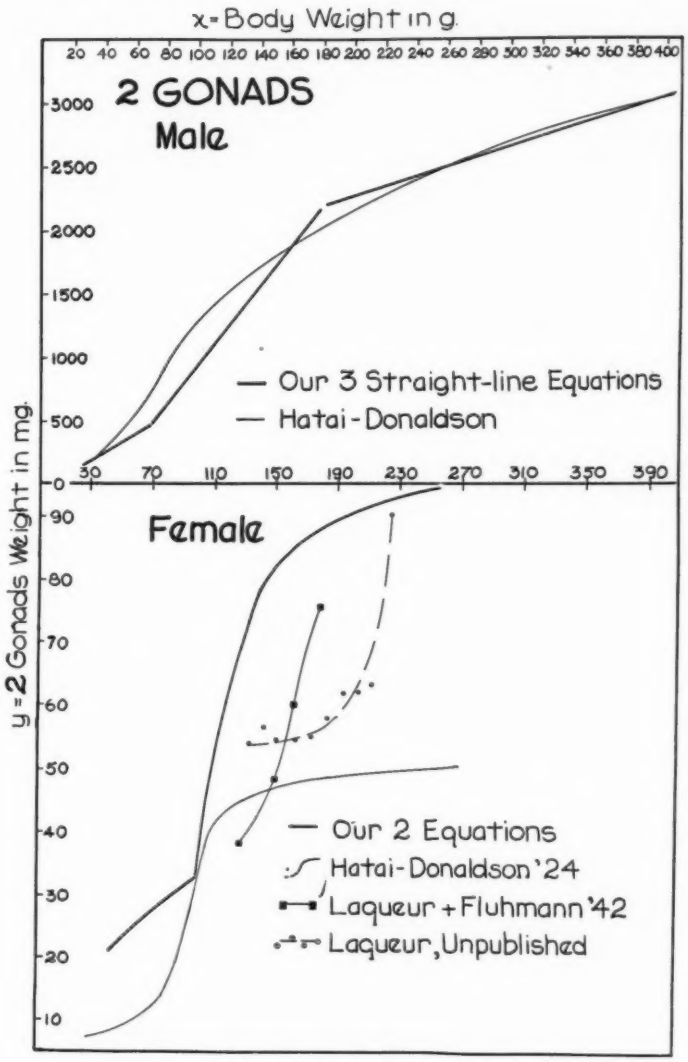
x = Body Weight in g.
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400
2 GONADS
Male
3000
2500
2000
1500
1000
500
0
— Our 3 Straight-line Equations
— Hatai-Donaldson
30 70 110 150 190 230 270 310 350 390
Female
90
80
70
60
50
40
30
20
10
y = 2 Gonads Weight in mg.
— Our 2 Equations
Hatai-Donaldson '24
Laqueur + Fluhmann '42
Laqueur, Unpublished

FIGURE 3

Summary

This is the third and last of three papers presenting tables for experimenters who have occasion to predict normal organ-weight in albino rats. The first dealt with the heart, kidneys and liver; the second with the suprarenals; and this finishes by converting the more complicated curves of gonad growth into convenient tables.

References

Backman, G. 1931. Das Wachstumproblem, *Ergebnisse der Physiologie,* pp. 883-973.

Bachman, G. 1939. Methodik der theoretischen Wiedergabe beobachteter Wachstumsserien, Lunds Universitets Ärsskrift, N.F. Avd. 2, Vol. 35, No. 8, pp. 1-20.

Growth, 1950, **14**, 107-110.

VARIATION IN THE NUMBER AND IN THE WEIGHTS OF THE FETUSES IN EACH LITTER IN A SERIES OF PUPPIES

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(Received for publication March 15, 1950)

A series of 156 fetal and 24 newborn puppies from dogs used in the Physiology Department is being studied. The growth of some of the organs and systems has already been published. The ages of some of these puppies is known, but most of them are of unknown age. Some of the organs of those of known age were plotted against age and also against body weight and body length and it was found that body weight and body length gave better curves with less scatter than the use of chronological age. While looking over some of the groups it was found that often members of the same litter would be quite widely separated when they were plotted against body weight or body length. This suggested the study of the variation in the weights of the specimens from each litter.

There have been several studies concerning the number of specimens per litter and also the variations in the average weights of the litters. These have been correlated with various conditions, such as age and other conditions of the mothers. King (1939) has reviewed much of this literature, and a paper by Ibsen (1928) gives a very careful and complete study of 113 guinea pig litters. So far as is known no attempts have been made to study the variation in weight of the specimens within each litter.

The 180 puppies came from 37 litters ranging from one to 9 per litter. Thirty litters of the fetal dogs ranged from one to 9 per litter with an average of 5.2 per litter. There were three litters of 8 each and three with 9 fetuses. The seven litters of newborn puppies ranged from one to 6 per litter with an average of 3.4 puppies. There were two litters with but one specimen each; one of fetal, and one of newborn puppies. All of these specimens were taken from dogs used for

experimental purposes in the Physiology Department and I wish to express my gratitude to the members of this department for permitting me to get this material. I also wish to express my gratitude to Dr. Robert L. Corder who collected many of these puppies.

This study was first suggested when it was found that in one litter of 8 fetuses, the largest fetus was 2.6 times the weight of the smallest specimen. The ratio of the largest to the smallest fetus in each of the 35 litters was then determined. The two litters with one each were not included, of course. These ratios ranged from 1.078 to a maximum of 2.605, with an average of 1.31. These ratios were plotted (not shown) on the number of specimens per litter and also on the average weight of the litter, and there were no observable relationships between the variation in the weights of the litter mates and either the average weight of the litter or the number in the litter.

To get a more accurate measurement of the variation in weight in each litter, the average percentage deviation from the average of the litter weight was determined. These average percentage deviations varied from 1.71 to 22.77%, with an average of 7.63%. These percentage deviations were plotted on the average body weights of the litter and also on the number of specimens in the litter as was done for the ratios, and again there was only a general scattering of the data, with no definite trend which could be discovered.

Four litters were composed of all males or all females and these had average percentage deviations of from 5.43 to 8.26, or an average of 7.31%. This is but 4.2% less than the average of all the litters, and so we may conclude that the sex difference of the specimens was not the cause of the variation in the individual weights. These mother dogs were all mongrels and of all sizes and the variation in the size of the two parents may have been inherited by the various members of the litter. In these mongrels, it is very likely that at least some of these litters may have been sired by more than one male and thus we would have superfecundation (Arey, 1946). This might mean additional variation in the expected size of the offspring.

The variations in the individual weights in these fetal dogs seemed large but when the data shown in Table 1 were collected, it was found that the variations in these mongrel dogs was not as large as that for some of the pure bred animals or for some human multiple births.

The weights of the individual guinea pig fetuses (Ibsen, 1928)

TABLE 1

VARIATIONS IN INDIVIDUAL WEIGHTS WITHIN THE LITTER SHOWING THE RATIO OF THE HEAVIEST SPECIMEN TO THE LIGHTEST AND THE AVERAGE PERCENTAGE DEVIATION FROM THE AVERAGE WEIGHT OF THE LITTER

	Ratio	Average percentage deviation
37 litters of fetal and newborn dogs	1.31	7.63
103 litters of guinea pigs, Ibsen, '28	1.36	8.61
Goat quintuplets, Patt, '38	1.59	14.44
Goat triplets, Patt, '38	1.25	9.51
Human multiple births		
Dionne quintuplets, MacArthur, '39	1.48	13.27
E. triplets, Sanders, '32	1.31	10.18
W. triplets, Sanders, '32	1.46	14.29
Johnson triplets, Lund, '33	1.05	2.12

were given, and from these data the ratios and average percentage deviations were calculated in the same manner as for the fetal dogs (Table 1). Ten litters of but one guinea pig each were not used. The ratios for the guinea pigs average 3.82% larger than the ratios for the dogs and the average percentage deviation of the guinea pigs is 12.84% greater than that of the dog fetuses. The ratios of all of these animals except the one litter of goat triplets were larger than the ratios found in the dogs, and the average percentage deviation of the dogs was the smallest of any of these animals.

The birth weights of the Dionne quintuplets (MacArthur, 1939) were studied in the same manner as the weights of the dog fetuses and the ratios and the average percentage deviations were found to be respectively 12.98 and 73.92% greater than these data for the dogs (Table 1). The only ratios and average percentage deviations of lower value than those found in the dogs, are those for the Johnson triplets and the ratio of the goat triplets.

Ibsen finds the smaller guinea pigs midway in the uterine horn and he also states that those fetuses most crowded had made the poorest gains. It was expected that the larger litters of these dogs would show the greatest variation in the individual weights but, as stated above, when these ratios and average deviations were plotted on the number per litter there was no consistent trend in the distribution of the data. The guinea pig average percentage deviations were plotted on number per litter and these like the percentage deviations of the dogs were very scattered but the larger percentage deviations were

found in the larger litters of guinea pigs, and although they were very irregular and very scattered, there was possibly a trend toward greater variability in the larger litters. The two greatest average percentage deviations of over 34% are found in litters of 4 and 7 specimens.

Two explanations are suggested to account for this variability within a litter, first a variation in the inherent growth tendency of the individual, which may or may not be due to variation in genetic structure, and second, a possible crowding or faulty implantation of the placenta, or a failure of the placenta to develop normally. A satisfactory explanation must await further study.

References

Arey, L. B. 1946. Developmental Anatomy, 5th Ed., Philadelphia, W. B. Saunders Co.

Ibsen, H. L. 1928. Prenatal growth in guinea pigs with special reference to environmental factors affecting weight at birth. *J. Exp. Zool.*, **51**, 51-91.

King, Helen Dean. 1939. Life processes in gray Norway rats during fourteen years in captivity. *Am. Anat. Mem.* #17.

Lund, S. E. T. 1933. A psycho-biological study of a set of identical triplets. *Human Biol.*, **5**, 1-34.

MacArthur, J. W. 1939. Genetics in quintuplets. II. Trends of growth in the Dionne quintuplets. *J. Heredity*, **30**, 359-364.

Patt, R. F. 1938. Dionnes of the goat world. *J. Heredity*, **29**, 17-20.

Sanders, J. 1932. Similarities in triplets. A contribution to the knowledge of heredity in triplets. *J. Heredity*, **23**, 225-234.

Growth, 1950, **14**, 111-112.

BOOK REVIEW

The Chemistry and Physiology of Growth. Edited by Arthur K. Parpart. Published by Princeton University Press. 1949. $4.50. Contains essays by 10 of the outstanding contributors to the field. Resulting from the 1946 Princeton Convocation on Growth.

REVIEWED BY F. S. HAMMETT

This collection represents above all else a tremendously satisfying awareness on the part of these explorers that: growth is not single but multiple; the integrated expression of the basic activities of initiation, proliferation, differentiation, organization and constructive increase; as conditioned by the non-growth activities of maintenance, regression, and catabolism; and the four basic attributes of direction, substance, state of substance, and form peculiar thereto as comprised in the disciplines of genetics, chemistry, physics, and anatomy (cytology). In other words that there are no boundaries in science; only interdependencies. Concepts which have been preached almost *ad nauseam* by this reviewer from 1936 onward.

Another outstanding aspect of these presentations is the absence of dogmatism. While the authors may state their precepts in what may seem irreversible terms, nonetheless each and every one exhibits a mind receptive to change. They are not exclusionists. And they admit in the words of Chas. Fort, that "everything in our experience is only a part of something else that in turn is only a part of still something else." Thus the barriers are being disposed of. And teamwork in attempting to solve the mysteries of the most general property of all things is developing.

Much yet is of course needed. We need to know whether or not the con-joining of amino acids into protein as the reaction-base of living and growth is by other than the classical Fisher model. We need to know how and to what effect amino acids are conjugated in protein with such as lipids and carbohydrates. It could be such is dissolved in isolation. And their importance to the progress and energy supply of growth is undetected. And we need to know which naturally-occurring chemical groups and/or amino acid as constellation thereof, is specific to such specific activities as shift of cells from no-

growth to growth, cell division, cell specialization, cell segregation, and cell increase in substance. While two such have been identified as probable (SH for proliferation; some group carried by proline and its analogs for cell specialization); only hints are available for the rest. But that's the fun of being a scientist. You no sooner open one door than a hundred others present themselves to be explored.

Every worker in the field of growth who also thinks about growth as something other than a waste-basket word into which he can throw his ignorance will profit by owning, reading, and thinking about this collection of essays. Only the unendowed will fail to sense its contribution to the foundations of the unity of science.

Growth, 1950, **14**, 113-121

DEVELOPMENTAL EFFECTS OBSERVED IN HABROBRACON AFTER EXPOSURE TO BETA RADIATION

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INTRODUCTION

The major effects of radiation on developing structures are the retardation or inhibition of growth and differentiation and the production of atypical organs. This thesis is made abundantly clear in the comprehensive review of Butler (1936). Considerable attention has been paid to the effects of X-radiation, emanations of radium, and ultra-violet radiation on embryonic tissue. The preliminary paper of Martin, Dent, and Joseph (1948) reported the presence of morphological abnormalities among specimens of the parasitic wasp, *Habrobracon juglandis* (Ashmead), which had been exposed to radioactive phosphorus throughout development. Recently Blumel (1950) published findings from a similar investigation in which specimens from 2 species of *Drosophila* were studied after continuous exposure to P^{32} during the developmental period. Again, offspring exhibiting morphological abnormalities were seen and, also, genetic mutations were produced.

H. juglandis has been the subject of a considerable amount of investigation involving the effects of X-radiation (Martin, 1947), but these studies have been made largely with the production of genetical mutations as the end in view and have considered, almost exclusively, the irradiation of unlaid eggs. The present study is concerned with the effects obtained by the subjection to varying amounts of beta radiation of individuals in the early stages of parthenogenetic development.

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Materials and Methods

Indebtedness to Dr. A. R. Whiting of the University of Pennsylvania, to the personnel of the Radiation Laboratory of the University of Pittsburgh, and to Dr. Albert Martin, Jr. of Mount Mercy College is gratefully acknowledged. The animals used in this investigation were specimens of *H. juglandis* from wild-type stock 33 which was kindly supplied by Dr. Whiting. The radioactive phosphorus (P^{32}) was furnished by the Radiation Laboratory of the University of Pittsburgh. Dr. Martin gave invaluable advice regarding the culturing of the wasps.

The larvae of *H. juglandis* are parasitic on the larvae of the Mediterranian Flour Moth, *Ephestia kuehniella* (Zeller). The usual method of culturing this wasp is set forth in detail by Martin (1947). In general, it involves putting female wasps in cotton-stoppered shell vials with caterpillars of *E. kuehniella*. The female wasps sting the caterpillars to immobility. The eggs of the wasps are then deposited on the caterpillars and the wasp larvae feed upon the caterpillars from the time of emergence from the eggs until the beginning of pupation. The onset of pupation is marked by the spinning of silk cocoons about themselves by the wasp larvae. Imago wasps eclose from the cocoons. The adult wasps are transferred to other vials, new caterpillars are added, and so on for each succeeding generation. During the present investigation the vials containing the wasps and/or their young were kept in an incubator the humidity of which was high and the temperature of which varied between 29° and 30° C., the optimum temperature range recommended by Martin. The vials were removed from the incubator only for brief periods of time during which observations were made and experimental manipulations carried out.

In this study the conventional culture method was modified somewhat to permit and facilitate the exposure of developing individuals to the emanations of radioactive phosphorus. Three sorts of vials were used: (a) *Culture vials* in which the eggs were actually laid and cultured. These were shell vials with an inside diameter of 18 mm., the bottoms of which had been removed and replaced by a layer of thin cellophane (3.2 mg./cm.^{2} of surface area). (b) *Experimental exposure vials* which were shell vials having an inside diameter of 20 mm. and which were encased except for their open mouths, in lead jackets with walls 4 mm. thick. In preparing these vials for use they

were filled to a depth of 4 mm. with aqueous solutions of P^{32} in the form of $H_3P^{32}O_4$ (Specific activity—0.025 mg. P^{31}/mc., total solids—0.009-0.500 mg./ml.). The water was then evaporated off leaving the radioactive material deposited on the glass. Checks made with a Geiger counter showed that no radiation escaped through the lead jackets. (c) *Control exposure vials* which were identical with the experimental exposure vials except that they contained no P^{32}.

Experimental Procedure

Virgin female wasps which had eclosed less than 5 days previously were put in culture vials (one to a vial) and to each culture vial were added 4 caterpillars. The culture vials were stoppered with cheesecloth-covered plugs of cotton, and were then put in the incubator for 48 hours during which time eggs were laid. In the course of the laying period each culture vial was from time to time checked to make certain that the caterpillars, after they had been immobilized, were lying flat on the cellophane; thus the eggs were laid in rather close proximity to the cellophane.

The vials were grouped in pairs so that each pair contained wasps that were sisters. At the conclusion of the 48 hour laying period the adult wasps were removed with care so as not to disturb the eggs and one of each pair of vials was then fitted into an experimental exposure vial and one into a control exposure vial. Thus the progeny of one of each of the pairs of virgin sister wasps was irradiated through the cellophane while the progeny of the other provided control specimens. The arrangement of the vials is illustrated in the sectional diagram, Figure 1. The culture vials containing the eggs

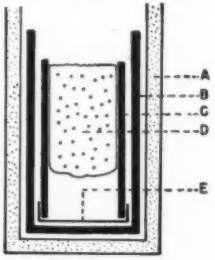

FIGURE 1

Sectional diagram showing arrangement of vials during exposure period. *A*—lead jacket, *B*—exposure vial (control or experimental), *C*—culture vial, *D*—cotton plug, and *E*—cellophane.

and/or young larvae (Under the conditions of this investigation the eggs of *H. juglandis* are transformed into larvae within 48 hours.) were incubated within the exposure vials (both experimental and control) for 48 hours. This period of time was termed the exposure period. At the beginning of each exposure period the P^{32} in the experimental exposure vials was at some known level of activity in the range between 68 μc. and 2,100 μc. Means for measuring or calculating in equivalent roentgens the radiation dosages received by the developing individuals were not available but in addition to the initial activities of the radiation sources the following facts are known: the mass of cellophane was 3.2 mg./cm.^{2} of surface area (one side). Most of the eggs were deposited on the lateral or ventral surfaces of the caterpillars and were exposed at less than 2 mm. from the source of radiation. A few laid on top of the caterpillars were 2-3 mm. from the source of radiation and were somewhat shielded from it by the bodies of the caterpillars. The long axis of the wasp egg measures approximately 600 μ and the short axis, approximately 150 μ.

With the termination of the exposure period the culture vials were removed from their respective exposure vials and incubation was continued. Records of daily observations were kept until the developing individuals either died or emerged as adults.

Results

Table I gives a listing of the mean numbers of larvae, pupae, and imagoes which appeared in the culture vials exposed at various levels of radiation.

At the three highest levels of P^{32} activity the mean number of larvae per vial was less than half the mean number which appeared in the control vials. The larvae which had been exposed to sources of beta radiation with initial activities of 2,100 μc. grew only to about one half of the typical size and were all dead within 6 days following the beginning of the exposure period. The larvae of all the remaining vials, in general, attained full larval growth; however, the relatively small numbers of larvae in the vials exposed at the range, 1,200 μc. to 2,100 μc., indicates that around half the eggs laid in those vials did not hatch.

Control larvae underwent pupation after 3 to 5 days following the beginning of the exposure period. In the experimental vials which

TABLE I

RELATION BETWEEN LEVEL OF BETA RADIATION AND DEGREE OF DEVELOPMENT IN *Habrobracon*

Table shows the mean number of individuals per progeny which developed to larval, pupal, and imaginal stages, respectively, after exposure to P^{32} sources of various initial activities for periods of 48 hours during embryonic and/or very early larval stages.

Initial Activity of P^{32} in μc.	No. of Progenies Exposed	Dev. to Larval Stage	Standard Deviation	Dev. to Pupal Stage	Standard Deviation	% sans Cocoons	Dev. to Imaginal Stage	Standard Deviation
2100	4	9.0	4.9	None	—	—	None	—
1900	8	12.5	4.3	None	—	—	None	—
1600	8	17.0	8.3	None	—	—	None	—
1200	12	15.0	10.9	9*	—	77.8	None	—
1000	12	25.5	5.3	7.2	5.1	61.1	13*	—
800	12	19.3	7.1	6.3	3.6	88.9	5*	—
600	8	28.0	4.5	12.1	4.4	53.7	5*	—
400	12	28.5	4.6	20.4	5.9	25.9	17*	—
200	12	28.7	7.4	27.0	8.0	5.2	19.0	5.4
100	12	29.5	8.0	27.4	8.0	4.6	23.4	8.6
68	8	28.2	7.5	26.6	7.4	6.1	23.8	8.5
Control	108	33.4	8.9	33.4	8.9	<.01	32.1	8.7

*Total number not mean number.

had been exposed to P^{32} of 400 or more μc. of radioactivity this period was sometimes extended to 7 or 8 days, but for the most part there was apparently little retardation of the onset of pupation when it did take place. Pupal development was completely inhibited at 1,600 μc. and above and the number of pupae per vial at 1,000 μc., 800 μc., and 600 μc. was less than half the number typical to the controls. At 200 μc. and below, the control condition was approximated with respect to the numbers of larvae which underwent pupation. Those larvae which failed to pupate lived from 4 to 8 times the usual larval life span. They moved sluggishly and assumed a brown coloration which became progressively deeper until death ensued.

Eclosion from the cocoon took place among the control animals 7 days following the start of the exposure period. Those irradiated pupae which did not eclose after 3 weeks were assumed to be dead and were carefully examined. In none of the animals taken from vials exposed to 1,200 and 1,000 μc. were adult organs differentiated. Since under the conditions of these experiments, imaginal form is attained after about 36 hours of pupation, it is clear that development stopped in these experimental animals prior to that time. About half the pupae taken from vials which had been exposed to P^{32} sources

with initial activities of 800 μc. and 600 μc. had fully formed adult structures, and nearly all those irradiated at still lower levels of activity had apparently completed development before death.

Eight of the control individuals developed into adults without spinning cocoons; however, in the experimental series many of the wasps developed in this fashion (Table I). The ejection of faeces begins typically during the second day of pupation in *H. juglandis* (Martin, 1947). This process was used as a criterion for estimating the beginning of the pupal period for the cocoon-less pupae. Whereas imagoes from cocoons (both control and experimental individuals) were fully formed and eclosed from the cocoon 7 days following the onset of the exposure period, the cocoon-less animals took on full imaginal form only after 10 to 13 days. The latter were apparently normal in the body region but their appendages tended to stick to their bodies and their wings were usually wrinkled or unexpanded. Their motions were confined to feeble movements of the head and abdomen. None survived more than a few hours after attaining imaginal form.

In culture vials exposed to P^{32} sources with initial activities between 400 μc. and 1,000 μc. there were only occasional pupae which developed into imagoes and when the source activity was above 1,000 μc. there were none at all. Approximately 2/3 of the pupae from vials which had been exposed to 200 μc. attained adult form (Table I). At 100 μc. and at 68 μc. the numbers of adults found in the vials approached more closely the numbers found in the control vials.

Of the experimental animals 17.6% were found to exhibit morphological abnormalities (fused ocelli, atypical antennae, and wrinkled or unexpanded wings, with frequency of occurrence in the order named), but the same abnormalities occurred in 14.2% of the control specimens and statistical analysis shows the two percentages not to be significantly different. However, twenty-five of the apparently normal experimental progeny (all males, since they developed from the eggs of virgin females) were selected at random and put into vials with caterpillars and virgin female wasps from stock cultures. Although most of these male-female pairs were observed to engage in mating actions, all offspring were exclusively male; giving indication that the males from the experimental vials were sterile. It is anticipated that this indicated developmental effect will be fully investigated in future work.

Discussion

In general there was a definite correlation between the amount of radiation released in terms of the activity level of the radiation source at the beginning of the exposure period and the extent of mortality among the irradiated individuals; also between the amount of radiation and the developmental stage at which death usually occurred. If one assumes that the numbers of viable eggs laid in the culture vials which were later irradiated and the numbers of viable eggs laid in the control culture vials were approximately the same, then one can conclude that death almost always occurs in the egg or during the larval stage when *H. juglandis* is irradiated under the conditions of this investigation with P^{32} sources at initial activities of 1,200-2,100 μc. Further, it can be concluded that development is arrested before hatching or early in the larval period at 2,100 μc. Halberstaeder, Goldhaber, and Hecht (1943) observed similar effects in *Calliphora* larvae which had received 300,000-2,000,000 roentgen units of X-radiation and in *Culex* larvae which had been irradiated with 30,000-2,000,000 roentgen units of X-rays. They found that imagoes did not develop when irradiated above the 5,000 roentgen level in *Calliphora* or above the 8,000 roentgen level in *Culex*. In the present investigation adults did not develop when radiation came from P^{32} sources with initial activities of 1,200 or more μc. and appeared rarely above 200 μc.

Hassett and Jenkins (1949) and Bugher and Taylor (1949) in reporting techniques for marking mosquitoes with P^{32} have commented briefly on developmental effects seen in *Aëdes aegypti* the larvae of which had been cultured in solutions of $NaHP^{32}O_4$. The latter authors found that larvae never reached the pupal stage when cultured in baths containing P^{32} at radioactivities "still higher than 200 mμc. per mosquito." Hassett and Jenkins state that all larvae were greatly retarded in rate of development when reared in solutions ranging in activity strength from 0.05 to 10 μc./ml. and that at the higher concentrations of this range pupation did not occur for over 20 days.

Decreases in the rate of development were observed in those specimens of *H. juglandis* discussed herein which continued to develop after irradiation with sources having initial activities of 400 μc. or more. In those animals the onset of pupation was often delayed

2 to 3 days and those few which reached the imaginal stage took from 2 to 6 days longer in doing so than did the control animals.

Blumel (1950) cultured pairs of *Drosophila virilis* and of *Drosophila melanogaster* on a medium to which radioactive H_3PO_4 had been added. No comment was made on rates of development but the progenies which hatched from the *D. virilis* matings were small in number while the *D. melanogaster* progenies contained the typical compliment of individuals.

As was stated in the preceding section occasional morphological abnormalities did occur among the experimental animals but not in significantly greater proportion than among the controls and probably were the expressions of factors inbred in the stock. While Martin, Dent, and Joseph (1948) reported an unusual number of abnormalities which apparently resulted from the exposure of specimens of *H. juglandis* to P^{32}, these specimens were irradiated as unlaid eggs and throughout the entire developmental period were in direct contact with the radiation source. Those same conditions obtained also for the previously cited results of Blumel. Further, her specimens consumed food containing P^{32} and were thus subjected to radiation from both internal and external sources.

Relatively high percentages of cocoon-less pupae were found at those radiation levels which usually brought about death during pupation (Table I). Above the 200 μc. level imagoes rarely appeared and there is a sharp rise in the percentage of pupae without cocoons. Animals without cocoons, however, apparently completed pupal development as readily as did animals with cocoons.

That indications of sterility were seen in animals which were otherwise apparently normal is not at all surprising. Bugher and Taylor (1949) noted that when reared in P^{32} baths at a radioactivity of "200 mμc. per mosquito", the adults of *Aëdes aegypti* failed to produce eggs. Also, these authors failed to find ovaries in such mosquitoes. Similarly: Blumel (1950) found that among 21 female and 17 male specimens of *Drosophila virilis,* survivors of 25 progenies which had been cultured on a P^{32}-containing medium, only 7 females were fertile. In *H. juglandis* germ cells are visibly differentiated from somatic cells in early cleavage (Speicher, 1936). It is easily conceivable that this differentiation might include or produce a differential susceptivity to radiation damage.

Summary

1. Developing individuals of *H. juglandis* were exposed to various levels of beta radiation for 48 hour periods just before hatching or during the early larval period.

2. At the highest levels of radiation used the wasps died before hatching or in the larval stage. At intermediate levels death usually occurred during pupation with a considerable degree of inverse correlation between the extent of imaginal differentiation and the amount of radiation given off. At the lowest levels it was apparent that most of the animals attained adult form.

3. The rate of development was somewhat reduced at the highest and intermediate levels but not at the lowest levels.

4. Pupation without cocoon formation was a frequent occurrence at the intermediate levels.

5. Evidence which indicates that irradiated animals reaching adulthood were sterile is presented.

References

1. Blumel, Johanna. 1950. Action of radioactive phosphorus in *Drosophila*. *Science*, 111:205-206.
2. Bugher, J. C., & Taylor, M. 1949. Radiophosphorus and radiostrontium in mosquitoes. Preliminary Report. *Science*, **110**:146-147.
3. Butler, E. G. 1936. The effects of radium and X-rays on embryonic development. Biological Effects of Radiation, Mc-Graw-Hill Book Company, Inc., New York.
4. Halberstaedter, L., Goldhaber, G., & Hecht, O. 1943. The effect of X-rays on development of insects. *Growth*, **7**:413-426.
5. Hassett, C. C., & Jenkins, D. W. 1949. Production of radioactive mosquitoes. *Science*, **110**:109-110.
6. Martin, A., Jr. 1947. An introduction to the genetics of *Habrobracon juglandis Ashmead*. Hobson Book Press, New York.
7. Martin, A., Jr., Dent, J. N., & Joseph, L. 1948. Effects of beta rays on *Habrobracon juglandis*. *Genetics*, **33**:619.
8. Speicher, B. R. 1936. Oogenesis, fertilization, and early cleavage in *Habrobracon*. *J. Morph.*, **59**:401-421.

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THE GROWTH OF INBRED AND HYBRID MAIZE[1]

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Practically all attempts to define the nature of hybrid vigor have dealt with the phenomenon in terms of size factors. Consideration of these factors is of primary importance even though it must be recognized that the hybrid advantage often involves factors other than those having to do with size. While geneticists were dealing principally with theoretical units of inheritance on one end of the scale and mature characteristics on the other, it was simple to conceive of size differences in terms of so many grams of weight or so many centimeters of height and to relate the inheritance of such differences to a gene that added a number of grams of weight or a number of centimeters of height either in absolute terms or in terms of a percentage. As investigators became more interested in the developmental processes by which the genes control the expression of characteristics, it became increasingly apparent that interpretations of size differences had to be based upon the elements of initial size, rate of growth, and the duration of the growth period; and that genetic interpretations had to deal with these considerations rather than with specific genes adding determined amounts of material.

We are now progressing rapidly toward the point where we can deal with size differences in terms of the time and rate of production of enzymes, vitamins, and other growth substances, and in terms of the level of efficiency of various metabolic processes. Before we can reach this point, however, we must have a good deal more sign-post information relative to the growth cycles of the organisms which we are studying. It is toward this end that this particular experiment was designed.

The relation of growth factors to the problem of hybrid vigor has

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been discussed in detail in the review papers of Smith (1944) and Whaley (1944). Jones' hypothesis relating heterosis to the presence in the hybrid of a combination of linked dominant genes was put forward in 1917 and until 1930 was generally accepted without much question as to the manner of operation of such genes. In that year Ashby began publication of a series of papers on studies of the inheritance of so-called physiological characters and the relationship of their inheritance to the development of hybrid vigor.

Ashby's first study (1930) was on maize and covered the period of 20 to 70 days after germination. His data indicate that the vigorous hybrids had the same relative growth rate as the faster growing parent and that the hybrids had no advantage either in duration of the growing period or in factors of cell size or photosynthetic efficiency of the leaves. The hybrids did have greater initial embryo weights and the hybrid seed showed a higher percentage of germination. From this particular study Ashby drew the indicated conclusion that the hybrid owed its advantage to its larger embryo. Such embryo size advantage has been shown to exist in several other maize hybrids (Murdoch, 1940). In a second paper Ashby (1932) reported further on maize crosses and noted that differences in embryo weight in reciprocal crosses could be detected as maturity differences when, as his evidence seemed to indicate, the hybrid inherited its grand period of growth rate from the faster growing parent as a simple mendelian dominant.

In 1935, Lindstrom designed what he supposed to be a test of Ashby's hypothesis that the hybrid's advantage was merely a reflection of an advantage in "initial capital." Although it did not constitute such a test, Lindstrom's experiment is of importance in relation to the development of size differences. His experimental procedure was to decapitate the F_1 seedlings of a heterotic cross and then to compare their growth with that of the parental strains. He was able to show that, despite the removal of much leaf tissue, the vigorous hybrids finally came to exceed the parental strains in terms of dry weight of both the plants and the ears.

East (1936) made a critical analysis of the relation of size factors to the heterosis problem and took Ashby to task severely for some of his conclusions. East listed several cases in which especially vigorous hybrids developed from seeds which were no larger than those of one of the parents and hence presumably had no greater "initial capital."

Studies of growth factors in relation to hybrid vigor in the tomato by Hatcher (1939, 1940) and Whaley (1939a and b) have eliminated either embryo size or size of the plumular meristem as of general importance in relation to hybrid vigor, despite the fact that Ashby (1936) and Luckwill (1937, 1939) had earlier related hybrid vigor in the tomato to these factors.

The present study is an attempt to analyze, in somewhat more detail than previously, the growth of various inbred lines of maize and their hybrids, to determine the characteristics of the individual growth patterns, and, if possible, to relate these characteristics to the development of hybrid vigor. The experiment was performed in two parts, one dealing with seedling growth under conditions which furnished no external supply of nutrients and the other dealing with the grand period of growth and the leveling of the growth curve as the plants approached maturity. To facilitate presentation, the data for each part are given separately.

Seed of the inbred lines and the hybrids was kindly furnished by the Texas Agricultural Experiment Station, College Station, Texas. The lines selected were chosen because the hybrids are particularly vigorous and are well-adapted to the conditions of moderate drought that frequently occur in central Texas during the corn growing season.

Seeds and Seedling Growth

Prior to planting, the seeds were sorted carefully and any injured or obviously atypical seeds were eliminated. One hundred seeds of each line were then selected at random from the remaining seed and weighed individually. Embryos were dissected out of 20 seeds selected at random from this lot of 100. The scutellum was removed from the rest of the embryo and each primordial axis was then weighed individually. This excision procedure obviously involves the possibility of error related to the uniformity of the removal of the scutellum. Care was exercised to make the operation as uniform as possible and as the figures indicate the variation among embryos so excised was not great.

Table 1 gives air dry seed and embryo weights for the inbreds and the various hybrids. The seed weight of the inbreds ranges from 172 ± 5.4 mg. for line 851 to 289 ± 5.1 mg. for line 855. Embryo weights range from 2.3 ± 0.2 mg. for lines 851 and 852 to 11.2 ± 0.5 mg.

TABLE 1
MEAN AIR-DRY SEED AND EMBRYO WEIGHTS

	Seed Weight mg.	Embryo Weight mg.
850	193±6.3	8.7±0.2
851	172±5.4	2.3±0.2
852	176±3.4	2.3±0.2
853	243±7.2	2.6±0.3
854	239±7.2	4.8±0.5
855	289±5.1	3.8±0.4
856	180±6.2	7.1±0.4
857	219±8.8	11.2±0.5
858	259±7.4	8.2±0.5
859	192±5.6	4.9±0.3
850 x 853	278±6.3	3.3±0.3
850 x 857	283±5.3	9.9±0.3
852 x 851	196±5.3	3.4±0.3
852 x 859	128±1.7	2.2±0.1
853 x 857	254±6.1	4.3±0.3
854 x 850	255±8.6	3.2±0.4
854 x 853	208±6.1	3.4±0.3
854 x 857	227±3.9	3.6±0.2
855 x 856	306±12.5	4.4±0.3
858 x 856	278±4.6	9.8±0.3
858 x 859	294±6.8	6.3±0.2
(850 x 853) x (854 x 857)	315±6.4	3.3±0.2
(852 x 851) x (855 x 856)	279±4.6	4.2±0.3
(852 x 859) x (855 x 856)	339±8.8	4.5±0.2
(858 x 859) x (855 x 856)	431±6.7	4.5±0.2

The seed weights are given as the mean weights of 100 seeds, with the standard error. The embryo weights are given as the mean weights of 20 embryos, with the standard error.

for line 857. As a group, the seed weights of the single-cross hybrids are not much greater than those of the inbreds, nor do these hybrids appear, as a class, to have any embryo weight advantage. The seeds of the double-cross hybrids do have significantly greater weights, except in one instance, and the embryo weights are rather more uniform than those of the inbreds or the single-cross hybrids. The behavior of seed weight and embryo weight in the crosses leading to the formation of double-cross hybrids is best presented by considering the different combinations.

The cross 850 × 853, a low seed weight, high embryo weight inbred crossed with a medium seed weight, low embryo weight inbred gives a hybrid characterized by a somewhat higher seed weight than either parent and an intermediate embryo weight. The cross 854 × 857, a medium seed weight, medium embryo weight inbred by a medium seed weight, high embryo weight inbred, gives a hybrid with medium seed weight and medium embryo weight, both being intermediate to

those of the parental lines. Crossing the hybrids (850 × 853) × (854 × 857) results in increased seed weight, but no further increase in embryo weight.

Both 852 and 851 are low seed weight, low embryo weight types. Their hybrid shows some advantage as to both seed weight and embryo weight. The 855 × 856 hybrid has, perhaps, a slight seed weight advantage over its parents and an intermediate embryo weight. The double hybrid (852 × 851) × (855 × 856) is intermediate to its immediate hybrid parents as to both seed and embryo weights.

The cross 852 × 859 represents a low seed weight, low embryo weight inbred crossed with a low seed weight, medium embryo weight inbred. The hybrid seed weight is not significantly different from those of the parents and the hybrid embryo weight is approximately equal to that of 852, the smaller embryo parent. The cross 855 × 856 represents a medium seed weight, medium embryo weight inbred crossed with a low seed weight, high embryo weight inbred. The resulting hybrid has a fairly high seed weight and a medium embryo weight. Crossing the hybrids in this case yields a somewhat higher seed weight, medium embryo weight double-cross hybrid. This cross combination (852 × 859) × (855 × 856) differs from the others principally in the high seed weight of the double hybrid. This high seed weight is not accompanied by a high embryo weight.

858 × 859 is a cross of a medium seed weight, high embryo weight line with a low seed weight, medium embryo weight line. The hybrid has a medium seed weight and a medium to high embryo weight. Its seed weight is greater than that of either parent. Its embryo weight is intermediate between those of the two parents. Crossing 858 × 859 with 855 × 856 results in a considerable increase in the seed weight of the double cross above the seed weights of either single cross but the embryo weight of double cross is nearly identical with that of the smaller single cross hybrid 855 × 856.

Both seed weight and embryo weight are conditioned by the genetic constitution of the tissue involved and by the characteristics of the supporting maternal tissues. Any consideration of seed weight determination must also take into account the fact that the endosperm contains two sets of chromosomes from the pistillate parent and one from the staminate parent. Some knowledge of the relative effects of gene combinations and maternal influence can be obtained from a

consideration of the hybrids involving the same pistillate parent but different staminate parents.

Line 850 was the pistillate parent in two crosses: 850 × 853 and 850 × 857. In both cases the hybrid seed weight greatly exceeded that of the pistillate parent and also showed a significant increase over that of the staminate parent which in each instance had a seed weight higher than that of 850.

Line 852 was used twice as a pistillate parent. In one case, 852 × 851, the hybrid seed weight was slightly greater than that of either parent. In the other, 852 × 859, the hybrid seed weight was significantly less than that of either parent. Here all the inbreds had relatively low seed weights and this characteristic appears in the hybrids.

Line 854 was the pistillate parent in three crosses. 854 × 850 was a hybrid with a seed weight greater than that of either parent; 854 × 853 had a seed weight somewhat lower than that of either parent; 854 × 857 had a seed weight intermediate between those of the parents. Despite these variations, line 854 seems to produce hybrids which do not differ from it greatly as to seed weight.

Line 858 was crossed with 856 and 859, both of which have relatively small seeds. In each instance the hybrid seed weight is significantly greater than that of the pistillate parent.

As might possibly have been anticipated, more extreme combinations of seed and embryo weights tend to appear among the inbreds than among the hybrids. It is perhaps of significance that with respect to embryo weight, particularly, the double-cross hybrids do not differ greatly from each other. It will be noted later that the double-cross hybrids differed less from each other with respect to most of the characteristics studied than did the single-cross hybrids or the inbreds. A comparison of the inbreds and the single-cross hybrids presents a different picture with respect to embryo weights than with respect to seed weights. In two instances, 852 × 856 and 854 × 857, the hybrid embryos weigh less than the embryos of either of the parents. In three instances, 850 × 853, 855 × 856, and 858 × 859, the hybrid embryos are intermediate between those of the parents. In only one instance, 852 × 851, is there any indication of hybrid vigor in embryo size. Whether there is any significance in the fact that this occurs in the cross between the lines with the smallest seeds cannot be determined from these data. When the single-cross hybrids are compared

with the double hybrids, in the making of which they were used as pistillate parents, the situation is seen to be quite different. In every instance the seed weight of the double-cross hybrids significantly exceeds the seed weight of the single-cross hybrid used as a pistillate parent and in three of the four cases the double-cross hybrid seed weights are significantly higher than those of either single-cross parent. Obviously this situation cannot be explained on the basis of either simple maternal influence, or xenia. Further attention will be given to the greatly increased seed weight of the double hybrids in the course of the discussion.

As to embryo weights, the single-cross hybrids differ from one another much less than do the inbreds, and the single- and double-cross hybrids are, in general, nearly of the same weight. It can be noted that the double hybrids exhibit much more uniformity and that their embryo weights are either intermediate between those of the parents or approximate that of the heavier parent.

It is clear that seed size and/or embryo size may or may not be involved in the production of hybrid vigor. In some instances heterosis is manifest in the hybrid embryos and in some instances xenia is detectable in the hybrid seed; but in a number of hybrids which are much more vigorous than their inbred parents neither advantage in seed weight nor advantage in embryo weight is apparent. This fact emphasizes the importance of investigating the characteristics of growth and development during the early seedling stage in an attempt to discover at what period, and possibly by means of what mechanism, heterosis is manifest.

The following procedure was used in studying seedling growth. Three hundred seeds of each line were selected at random from previously sorted lots. These seeds were soaked in distilled water for four hours and then were planted one inch apart in flats of washed sand in the greenhouse on June 12, 1948. The flats were watered immediately after the planting and then daily throughout the course of the experiment. Forty-eight hours after planting and at 48-hour intervals thereafter 20 typical plants were selected for sampling. Any obviously atypical plants were removed as soon as their deviation from the normal pattern of development became apparent. At each sampling the selected seedlings were dissected so as to permit determination of the weight of the shoot, the root, and the seed residue. Fresh weights were obtained immediately after sampling. The various

samples were then oven dried to constant weight and dry weights taken. It had been determined by a preliminary investigation that under the conditions of this experiment, seedling growth exhausted the food reserves of the seed within a 12-day period and that after 12 days, or in some cases 10 days, there occurred a weight decrease in the shoot and the root. This being true, samples were taken only over the 12-day period.

Table 2 shows the fresh and dry weight changes of shoot and root, and the seed residue for the first 12 days after planting, for four inbred lines, 850, 853, 854, and 857, two single-cross hybrids, 850 × 853 and 854 × 857, and one double-cross hybrid, (850 × 853) × (854 × 857). Table 2 also shows the fresh and dry weights of the seed residues on the 12th (or 10th) day of seedling growth. These figures are given in terms of the percentage of seed residue weight on the second day. The second day was chosen because at this stage the seed has become hydrated but there has been little transfer of material from the seed to the growing seedling. In the inbreds, germination was much less complete than among the hybrids and much less uniform from one seed to another. For this reason some of the inbred data do not reflect weight increases from one sampling period to the next. On the whole, however, the data afford valid comparisons among the lines and hybrids. The technique employed may be illustrated by a study of the data for line 853 (chosen here because the germination of 850 was relatively slow and early growth was poor). Line 853 had a dry seed weight at 243±7.2 milligrams, an embryo weight of 2.6±0.2 milligrams. At the scheduled first sampling period, 2 days after planting, the shoots weighed 20 mg., the roots 42 mg. The seeds had absorbed enough water to raise their fresh weight to 341 mg. The average dry weight was 215 mg. The comparison of this dry weight figure with the original air-dry seed weight suggests that the moisture percentage of the original air dry seed was relatively low. Similar data were obtained for all the inbreds and hybrids listed in Table 1, but to keep the tabular material within reasonable limits data on the other plants have been omitted from the tables; where of significance certain of these have been included in the text.

In an effort to arrive at some measure of the rate of growth in relation to the utilization of food reserves in the seed, it has been assumed, as noted above, that in most instances seedling growth exhausts these food reserves under these experimental conditions in a

TABLE 2

CHANGES IN WEIGHT (mg.) OF SEED, SHOOT AND ROOT DURING THE FIRST 12 DAYS OF SEEDLING GROWTH

		Fresh Weight				*Dry Weight*						
Strain	Days	Seed	Shoot	Root	Total Root and Shoot	Seed	Shoot	Root	Total Root and Shoot	Growth Factor	Depletion of Seed Material (Percent) Fresh	Dry
850	2	303				163						
	4	368				151						
	6											
	8	310	211	150	361	131	19	13	32	41		
	10	337	181	117	298	101	15	9	24	34		
	12	275	335	260	595	66	31	19	50	68	9	60
	4:12*											
	8:12**		63	58	61		61	68	64			
853	2	341	20	42	62	215	3	4	7	24		
	4	345	135	116	251	170	13	14	27	96		
	6	323	291	247	538	118	30	20	50	207		
	8	308	425	568	993	80	42	45	87	382		
	10	526	584	792	1376	64	53	63	116	529		
	12	264				57					23	73
	4:12*		23	15	18		25	22	23			
	8:12**		73	72	72		79	71	75			
854	2	372				221						
	4	331	143	131	274	146	15	14	29	57		
	6	327	379	320	699	95	43	27	70	146		
	8	335	491	517	1008	86	55	49	104	210		
	10	369	660	937	1597	57	65	68	133	333		
	12	304	836	1066	1902	47	82	62	144	396	18	79
	4:12*		17	12	14		18	23	20			
	8:12**		59	48	53		67	79	72			
857	2	383	19	26	45	238	3	3	6	4		
	4	298	141	152	293	132	15	17	32	26		
	6	292	416	253	669	106	42	22	64	60		
	8	232	570	517	1087	53	55	41	96	97		
	10	320	651	476	1127	63	62	53	115	101		

TABLE 2 (*continued*)

Strain	Days	*Fresh Weight*				*Dry Weight*					Depletion of Seed Material (Percent)	
		Seed	Shoot	Root	Total Root and Shoot	Seed	Shoot	Root	Total Root and Shoot	Growth Factor	Fresh	Dry
	12	330	772	750	1522	43	74	56	130	135	14	81
	4:12*		18	20	19		20	30	25			
	8:12**		74	69	71		74	73	74			
850 x 853	2	437				241						
	4					157	21	18	39			
	6	326	418	232	650	103	42	15	57	197		
	8	267	534	760	1294	61	57	56	113	392		
	10	287	734	676	1410	48	71	54	125	427		
	12	208	1018	868	1886	33	104	70	174	572	52	86
	4:12*						20	26	22			
	8:12**		52	88	69		55	80	65			
854 x 857	2	309	25	39	64	182	4	4	8	18		
	4	336	232	243	475	135	24	23	47	132		
	6	316	428	323	751	71	43	22	65	209		
	8	319	669	815	1484	51	75	60	135	412		
	10	280	657	811	1468	31	69	68	137	408		
	12	213	827	877	1704	26	87	73	160	473	31	86
	4:12*		28	28	28		28	32	29			
	8:12**		81	93	87		86	82	84			
(850 x 853)	2	420	11	29	40	215	2	4	6	12		
x	4	444	243	261	504	191	26	27	53	153		
(854 x 857)	6	421	565	541	1106	128	61	42	103	335		
	8	390	678	891	1569	92	79	64	143	475		
	10	380	848	887	1735	61	99	87	186	526		
	12	304	1058	1176	2234	48	105	90	195	677	28	78
	4:12*		23	22	23		25	30	27			
	8:12**		64	76	70		75	71	73			

*Percentage 4th day is of 12th day.
**Percentage 8th day is of 12th day.

12-day period. Twelfth-day weights, or 10th-day weights in the cases where 12th-day weights were smaller and thus indicated earlier food exhaustion, were taken to indicate the maximum amount of growth supported by the seed nutrients. Fourth- and 8th-day weights were compared on a percentage basis with the maximum weights attained on the 12th- or the 10th-day. In the case of line 853, in terms of fresh weight, the shoot had completed 23 per cent of its seed supported growth on the fourth day and 73 per cent on the eighth day. The root had completed 15 per cent on the fourth day and 72 per cent on the eighth day. In terms of total fresh weight, 18 per cent was complete on the fourth day and 72 per cent on the eighth day.

The figures on residual seed weight presumably reflect a movement of stored food materials into the growing shoot and root. The fresh weights of residual seed materials vary greatly, probably reflecting the difficulties of sampling this type of material on a fresh weight basis, but perhaps also reflecting differences in susceptibility to hydration associated with differences in composition and the changes in composition. To provide another figure for the comparison of growth, a so-called growth factor has been calculated. This growth factor is obtained by dividing the total fresh weight of the seedling or plant at any given time by the initial embryo weight. It thus reflects the multiple by which the embryo weight has been increased at that particular time. On the second day after the planting the embryo weight of line 853 had been increased by growth 24 times; on the fourth day, 96 times; on the sixth day, 207 times; on the 8th day, 382 times; and on the tenth day, which represents maximum seed-supported growth in this line, 529 times.

Among the inbreds, line 850 was the slowest to germinate and had the lowest growth factor, 68, over the 12-day period. Despite this fact, on the eighth day it had achieved some 61 per cent of its seed supported growth in terms of fresh weight and 64 per cent in terms of dry weight. Among the inbreds, there was much variation as to the early growth pattern. On the fourth day, lines 854 and 858 had achieved only 14 per cent of their seed-supported fresh weight growth and 20 per cent and 19 per cent, respectively, of their dry weight growth. Line 859 had achieved 31 per cent of its fresh weight growth and 36 per cent of its dry weight growth. Line 852 had achieved 30 per cent of its fresh weight growth and 40 per cent of its dry weight growth. By the eighth day, line 852 had achieved 83 per cent of its

fresh weight growth and 88 per cent of its dry weight growth. The growth factors varied greatly at each of the sampling periods. Line 853 had the highest growth factor value, 529, at the conclusion of the experimental period. It is perhaps of significance that the high growth factors all occurred in low embryo weight lines, whereas the high embryo weight lines all had relatively low growth factors. This certainly seems to indicate that the so-called initial capital cannot be an all-important factor in determining final size or final yield.

One cannot escape the conclusion that the small tissue masses have a higher level of efficiency. Size of the seedling at the end of 10 or 12 days is, however, a product of the initial tissue mass and the protoplasm's efficiency in reproducing itself. At the end of the experimental period of seedling growth the largest plants were those of line 854, plants of which totaled 1902 mg., and line 858, plants of which totaled 1782 mg. The embryo weights of these two lines were 4.8 ± 0.5 and 8.2 ± 0.5 respectively, and the growth factors, 396 and 217.

Comparison of the seedling growth patterns of the inbreds and the single-cross hybrids, reveals several facts. Line 850 germinated slowly, had a low twelfth-day growth factor, 68, and achieved 61 per cent of its fresh weight, and 64 per cent of its dry weight, in the 8-day period. Line 853 germinated rapidly, had a high growth factor, 529, and had achieved 72 and 75 per cent of its fresh weight and dry weight growth respectively, on the eighth day. The cross between these two lines produced a hybrid which germinated more rapidly than 850, but not as rapidly as 853, had a higher growth factor than either of the parents and achieved 69 and 65 per cent of its fresh weight and dry weight growth, respectively, in the first eight days. Its behavior approached much more nearly that of line 853 than of line 850. This cross can be compared with 850×857 which involved the same pistillate parent and a staminate parent with a very different seedling growth pattern. Line 857 is a large-embryo line which germinated rapidly, achieved a high percentage of its growth early, but had a relatively low growth factor, 135. The hybrid showed some delayed germination, a medium percentage of growth in the first 8 days and a growth factor in excess of the better growing parent but still low. The behavior of this hybrid tends to suggest that the seedling growth characteristics of line 857 are not markedly different from those of line 850.

Line 852 occurs in two crosses. It is a small-embryo line which germinated relatively early, achieved more than 80 per cent of its seedling growth in the first 8 days and had a growth factor of 443. Line 851 has embryos of almost the same weight, made 30 per cent less of its growth in the first 8 days and had a growth factor of 330. The hybrid 852 × 851 has a larger embryo than either inbred, equaled 852 in percentage of growth achieved in the first 8 days, and was intermediate between 851 and 852 as to growth characteristics. The cross 852 × 859 also involves 852 as a pistillate parent. Line 859 has a significantly greater embryo weight than 852 while its seed weight is in the same range with that of 852. The embryo weight of the hybrid is approximately the same as that of the smaller embryo parent. The hybrid seedling growth resembled that of 852 in terms of total growth, the speed at which the various growth levels were achieved, and the growth factor, which is, if anything, somewhat higher than that of line 852.

Line 853 was used only once as a pistillate parent, with line 857. 853 is a small embryo line which grew relatively slowly during the first part of the seedling growth period but had a high growth factor, 529, at the conclusion of the period. Line 857, as has already been noted, has an especially large embryo and a relatively low growth factor. The hybrid is intermediate as to embryo size, grew somewhat more slowly than 853, and had an intermediate but relatively high growth factor.

The cross 854 × 850 is the only one involving 850 as a staminate parent. Line 854 is an intermediate embryo weight inbred. It grows slowly but has a relatively high growth factor. The hybrid has smaller embryos than either of the inbreds, a somewhat delayed germination as compared with both parents and a growth factor approaching that of 854. When 854 is crossed with 853, a low embryo weight, rapidly germinating, high growth factor line, the hybrid has an intermediate embryo weight, grows rather slowly, but has a growth factor in excess of either of the parents. The hybrid 854 × 857 shows a still different pattern. In this cross the embryo weight is less than that of the smaller parent, but the growth rate is rapid and the growth factor is high.

When the seed, embryo and seedling growth characteristics of the inbreds, the single-cross hybrids, and the double hybrids are compared, there do not seem to be any clear-cut instances of dominance

of the characteristics of one line or another. There is, rather, a blending of characteristics, which tends to produce hybrids of intermediate position with respect to considerations of initial size but with higher growth factors. There appears to be no relation between the size of the embryo and the growth rates or the amount of seed material which the embryo and seedling use during early growth.

Later Growth

For determination of growth and size factors from the seedling stage to maturity, plants were grown in the field in the summer of 1948. Plantings were made on April 25. Two hundred seeds of each line and each hybrid were planted one foot apart in rows four feet apart. Each line row was replicated four times in a randomized design along the outer borders of which were planted two buffer rows of a line not used in the experiment. As the plants emerged, any obviously abnormal or atypical plants were removed. Such removal may, of course, have some effect upon the growth of adjacent plants, but such an effect is also likely in the case of plants removed during the sampling. That the removal of any plants may modify the picture of variation obtained from an experiment is clear. Where relatively small numbers of plants can be sampled, however, the exclusion of those which may be damaged is desirable. Not more than a dozen such plants were removed from the entire experiment, hence the effect of their removal is not likely to have been significant. In sampling, plants were removed according to a pre-determined pattern so as to maintain, as nearly as possible, the same spacing in all of the rows at all times. The plants were cut off at ground level, and no attempt was made to sample the roots. It is probable that the cuts varied over at least one or two internodes, but the differences from one line to another were great enough so that this variation would not have been significant.

While the plants were still relatively small, samples consisted of 30 plants of each line taken at weekly intervals. As the size of the plants increased, the number of plants in a sample was reduced first to 20, then to 10, and finally to 5. While it would have been desirable to weigh the plants in each sample individually so as to have an accurate measurement of the amount of variation, the magnitude of the experiment precluded this and the plants had to be weighed on a sample basis, the size being calculated as the average of the plants

in a given sample. Fresh weight determinations were made immediately after harvesting whenever possible. Where delay of more than a few minutes was involved, plants were wrapped in moist paper for protection against evaporation. After the fresh weight was determined, the plants were dried to constant weight in an oven or electrically-heated drying box.

Both fresh and dry weights were plotted semi-logarithmically. The growth curves of one inbred line, 850, are shown in Figure 1. (To

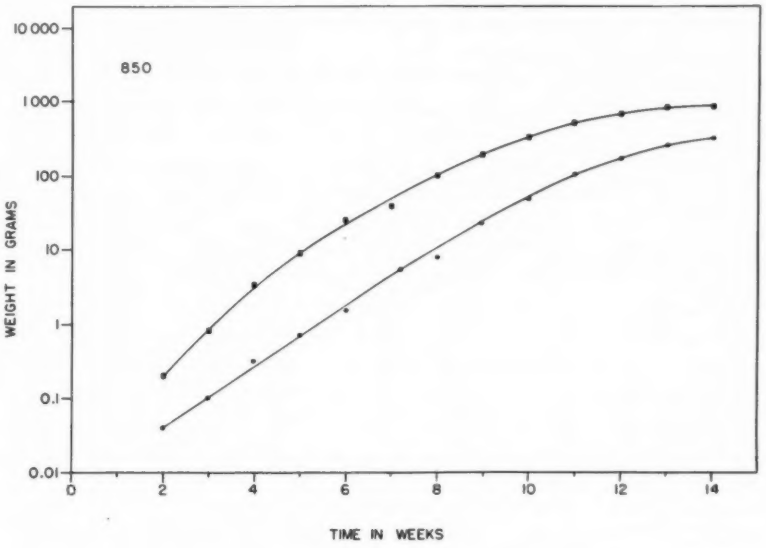

FIGURE 1. LINE 850

Curves showing fresh and dry weight increase of the shoots. The upper curve shows the fresh weight increase; the lower curve shows the dry weight increase.

conserve publication space, growth curves of the other inbred lines and the hybrids have been omitted, but the figures derived from them are included for inbreds 850, 853, 854, and 857, the single-cross hybrids 850×853 and 854×857, and the double-cross hybrid $(852 \times 853) \times (854 \times 857)$ in the tables given below.) From these curves calculations are presented to clarify some of the growth characteristics. First, the average weekly growth rates for the grand period

of growth and the period of growth decline accompanying maturation were calculated by the Blackman formula (1919): $W_n = W_0 e^{rt}$, where W_n is the size at any given time, W_0 is the size at the beginning of the growth period for which the calculation is being made, e is the base of the natural logarithm, 2.718, r is the growth rate, and t is the length of the time period. The r values were calculated for each inbred and hybrid and for both fresh weight and dry weight. These r values reflect, of course, only the degree of slope in the curves but their calculation makes it possible to detect differences which are very difficult to see in the curves themselves. Table 3 gives the r values from week to week.

To determine the r value for any week, a size measurement based on a sufficiently large sample must be available for the beginning of

TABLE 3

RELATIVE GROWTH RATES (*r* VALUES) FROM THE SECOND WEEK OF GROWTH TO MATURITY
(For method of calculation see the text)

	850		853		854		857	
Week	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
2	1.39	1.20	1.31	1.04	1.52	1.25	2.14	2.18
3	1.39	.92	1.22	1.08	1.36	1.25	1.85	1.79
4	1.10	.94	1.12	1.01	1.28	1.01	1.18	1.20
5	.92	.98	.83	.88	1.02	.93	1.07	.95
6	.77	.99	.74	.81	.79	.82	.85	.69
7	.65	.87	.57	.76	.52	.74	.76	.45
8	.59	.80	.48	.66	.34	.56	.63	.31
9	.58	.74	.32	.54	.18	.36	.44	.17
10	.45	.71	.09	.43	.04	.26	.27	.07
11	.34	.59	.04	.28	—	.11	.18	—
12	.16	.38	—	.17	—	—	—	—
13	.02	.22	—	—	—	—	—	—

	850 x 853		854 x 857		(850 x 853) x (854 x 857)	
Week	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
2			1.61	1.24		
3	1.61	1.26	1.66	1.39	1.72	1.54
4	1.26	1.26	1.27	1.20	1.25	1.31
5	.95	1.11	.88	1.04	.92	1.03
6	.69	.97	.69	.82	.62	.85
7	.55	.84	.59	.71	.52	.71
8	.39	.68	.43	.65	.42	.50
9	.32	.50	.34	.51	.32	.42
10	.24	.36	.13	.41	.21	.31
11	.13	.14	.06	.27	.06	.23
12	—	.04	—	.15	—	.15
13	—	—	—	—	—	.02
14	—	—	—	—	—	—

the week. In some of the lines such a sample was not available at the beginning of the second or third weeks. No significance should be attached to the absence of figures in the beginning of the growth period for it indicates only that a satisfactorily large sample was not yet available because of delayed germination or slow early growth, not that there was absence of growth.

Line 850 continued active growth, so far as the shoot is concerned, for a period of 12 weeks for both fresh weight and dry weight. The maximum fresh weight growth rate occurred from the second to the fourth week. (It is impossible to ascertain from the data available, whether the rate before the second week sampling period was greater or less than this value of 1.39). Line 850 showed a fresh weight growth curve of fairly gentle slope, the falling off of the growth rate thus being relatively uniform. Line 853 which was crossed to 850 in one of the hybrid combinations had an initial fresh weight growth rate slightly less than that of 850. The rate of falling off of its r value, that is, the rate of diminution of growth, was significantly more rapid and its total growth period was at least one week less than that of 850. In addition, the growth during the last two weeks of the active period was actually very little. The growth curve for 853 was thus significantly shorter than that for 850. The hybrid 850 $\times$ 853 had a higher initial fresh weight growth rate value than either of the inbreds. Its active growth period extended over 12 weeks as in the case of 853, but the shape of the curve resembles that of 850 somewhat more closely than that of 853.

The other inbred combination involved in this same series of crosses presented the following facts: 854 had an initial fresh weight growth value of 1.52 and its growth rate remained relatively high for at least 4 weeks. After this high rate period, falling off of the growth value was rapid and growth was completed within 11 weeks. Line 857 had the highest initial growth value detected in this series, 2.14. The length of its active shoot growth period was 12 weeks and the rate of falling off of the growth value was relatively slow. The hybrid 854 $\times$ 857 was intermediate as to both initial values and the apparent length of the fresh weight increase period. It is to be noted also that this hybrid had a higher r value for the third week of growth than for the second. The double hybrid (850 $\times$ 853) $\times$ (854 $\times$ 857) showed an initial r value somewhat higher than that of either of the single-cross hybrids, but otherwise its growth pattern was very similar to those of the two single-cross hybrids.

As would be anticipated, the r values for dry weight increase follow approximately the same pattern as those for fresh weight increase, but lag behind the fresh weight increase values in nearly all instances. In the case of 850 the initially detected dry weight r value was 1.20 in the second week, 0.19 lower than the fresh weight r value for the same period. During the next week the dry weight value was 0.92 but it increased again until during the sixth week it was 0.99. At this time the fresh weight increase value was 0.77. During the last week of active growth the dry weight r value exceeded that of the fresh weight by 0.20. In line 853 there is no sharp decrease followed by an increase in the early dry weight r values as there is in line 850. There is perhaps some significance in the fact that dry weight curves can differ in this manner in spite of the apparent similarity between corresponding fresh weight curves. This would seem to indicate the existence of different patterns of protoplasmic activity with the differences obscured by the rapid absorption of water associated with cell enlargement during certain phases of growth.

The hybrid 850 × 853 showed initially higher dry weight increases than either inbred but otherwise its dry weight growth pattern does not differ significantly from those of its parents. Line 857 differs from 854 in having a high initial r value. Also, its dry weight increase ceases before its fresh weight increase. The hybrid 854 × 857 resembles 854 much more closely than it does 857. The double-cross (850 × 853) × (854 × 857) does not show any significant differences from the single-cross hybrids, except for a somewhat higher initial r value.

A comparison of all of the inbreds and the hybrids showed that, in general, the hybrids had somewhat higher early growth rates and, at least during the first weeks, the decrease in rate was somewhat less abrupt in the hybrids than in the inbreds. In general, too, the hybrids completed the major part of their growth somewhat earlier than the inbreds though in most instances the hybrid growth cycle was not significantly shorter. Furthermore, as data to be presented later will show somewhat more clearly, the hybrids were more efficient than the inbreds in the production of dry weight material per unit of fresh weight material. The double-cross hybrids appeared to hold no significant growth rate advantages over the single-cross hybrids.

It is difficult to trace an influence of any particular inbred line in the hybrid combinations. This would be expected on the basis of the

TABLE 4

RATIOS OF FRESH WEIGHT INCREASE TO DRY WEIGHT INCREASE FROM THE SECOND WEEK AFTER PLANTING TO MATURITY. A FIGURE OF 1.000 REPRESENTS EQUAL RATES OF FRESH WEIGHT AND DRY WEIGHT INCREASE, VALUES GREATER THAN 1 INDICATE MORE RAPID FRESH WEIGHT THAN DRY WEIGHT INCREASE

Week	850	853	854	857	850 x 853	854 x 857	(850 x 853) x (854 x 857)
2	1.158	1.259	1.216	.982			
3	1.510	1.129	1.105	1.033	1.278	.988	1.116
4	1.170	1.109	1.267	.983	1.000	1.034	.954
5	.938	.943	1.096	1.126	.855	.934	.893
6	.785	.913	.963	1.232	.711	.822	.729
7	.747	.750	.702	1.689	.655	.784	.732
8	.737	.727	.607	2.032	.573	.709	.840
9	.783	.592	.500	2.588	.640	.627	.762
10	.633	.209	.154	3.857	.666	.240	.677
11	.576	.143	——	——	.928	——	.261
12	.410	——	——	——	——	——	——
13	.090	——	——	——	——	——	——
14	——	——	——	——	——	——	——

fact that growth rates obviously must be determined by a number of genes acting on different components of the metabolic processes.

Another revealing comparison is presented by the figures in Table 4. These figures represent the ratio of the rate of fresh weight increase to the rate of dry weight increase during each weekly period. They give some indication of the relative efficiency of the lines in synthesizing protoplasm.

Inasmuch as they are derived figures, no detailed comparison will be attempted but it is clear from the table that the single-cross hybrids formed a larger unit of dry matter per fixed unit of fresh weight somewhat earlier and, in most cases, more rapidly than the inbreds. The difference between the single-cross hybrids and the double-cross hybrids suggests again mostly increased uniformity of performance in the double-cross hybrids. The point at which the figure in each case falls below a value of one represents the time at which the processes of protoplasmic activity become dominant over mass increase of fresh weight involving a large amount of water absorption. As in most of the other characteristics of growth, the inbreds are exceedingly variable with respect to this point, and the combination of genetic constitutions in hybridization tends to iron out this variability.

To make possible a more detailed analysis of the growth differences a series of calculations relating to the rate and extent of growth has been based upon the growth curves. Line 850 (Fig. 1) will serve as

an example of the method employed in making these calculations. On the fresh weight curve the point at which the greatest shoot weight was reached was determined and the number of days growth represented by this point was recorded. For fresh weight of this line the maximum was 850 grams, reached in 92.4 days from planting. The growth period was then divided into 4 quarters based on the attainment of ¼, ½, ¾ and the total maximum fresh weight. Each of these weights was spotted on the growth curve and the time at which it was reached was determined.

Line 850 reached ¼ of its total fresh weight in 64.4 days, ½ in 74.2 days and ¾ in 81.9 days. The same set of determinations was also made for the dry weight increase curve. From these growth by quarter figures a growth factor was determined by dividing the growth during the quarter by the number of days in the quarter. The growth factor thus represents a calculation of the average amount of fresh weight material or dry weight material formed per day in each quarter. It will be obvious that for the first quarter a minor error is involved in this calculation. In making the calculation it has been assumed that all the material present at the end of the first quarter was formed during that period. No attempt has been made to take the weight of the shoot portion of the embryo into consideration. Inasmuch as all the measurements on these field-grown plants were made in grams and the embryo weights were all less than 12 milligrams the error involved is insignificant. These calculations of growth by quarters make it possible to compare the lines in several different ways and they reveal differences that could not be detected by over-all growth measurements.

Table 5 shows a comparison of growth by quarters of the inbreds and hybrids which enter into the (850 × 853) × (854 × 857) double cross.

TABLE 5

INCREASE IN FRESH WEIGHT AND DRY WEIGHT BY GROWTH QUARTERS TOGETHER WITH THE GROWTH FACTORS FOR EACH PERIOD AND THE PERCENT OF DRY MATTER AT MATURITY.

Size	Fresh		Dry		Size	Growth Factor	
	Days	Grams	Days	Grams		Fresh	Dry
				850			
1	92.4	840	98.0	310.0	Total Average	9.1	3.1
¾	81.9	630	89.6	232.5	¾-1	20.0	9.2
½	74.2	420	82.6	155.0	½-¾	28.6	11.1
¼	64.4	210	74.9	77.5	¼-½	21.4	10.1
					0-¼	3.3	1.0
				Percent Dry Matter at Maturity			
				36.9			

TABLE 5 (*continued*)

Size	Fresh Days	Fresh Grams	Dry Days	Dry Grams	Size	Growth Factor Fresh	Growth Factor Dry
				853			
1	81.2	660	91.0	228.0	Total Average	8.1	2.5
¾	69.3	495	80.5	171.0	¾-1	13.9	5.4
½	60.9	330	72.8	114.0	½-¾	19.6	7.4
¼	51.8	165	63.0	57.0	¼-½	18.3	5.8
					0-¼	3.2	.9
				Percent Dry Matter at Maturity 34.5			
				854			
1	72.1	560	83.3	145.0	Total Average	7.8	1.7
¾	60.9	420	71.4	108.8	¾-1	12.5	3.0
½	53.9	280	63.7	72.5	½-¾	20.0	4.7
¼	46.2	140	55.3	36.3	¼-½	18.2	4.3
					0-¼	3.0	.7
				Percent Dry Matter at Maturity 25.8			
				857			
1	77.0	840.0	84.0	400.0	Total Average	10.9	4.8
¾	62.3	630.0	73.5	300.0	¾-1	14.3	9.5
½	53.2	420.0	65.8	200.0	½-¾	23.1	13.0
¼	44.8	210.0	57.4	100.0	¼-½	25.0	11.9
					0-¼	4.7	1.7
				Percent Dry Matter at Maturity 47.6			
				850 x 853			
1	84.0	1600.0	86.1	480.0	Total Average	19.0	5.7
¾	72.1	1200.0	74.9	360.0	¾-1	33.6	10.7
½	63.0	800.0	67.2	240.0	½-¾	44.0	15.6
¼	51.8	400.0	59.5	120.0	¼-½	35.7	15.6
					0-¼	7.7	2.0
				Percent Dry Matter at Maturity 30.0			
				854 x 857			
1	82.0	1700.0	91.0	570.0	Total Average	20.8	6.3
¾	68.0	1275.0	79.8	427.5	¾-1	30.4	12.7
½	60.2	850.0	72.1	285.0	½-¾	55.2	18.5
¼	50.4	425.0	63.0	142.5	¼-½	43.4	15.7
					0-¼	8.4	2.3
				Percent Dry Matter at Maturity 33.5			
				(850 x 853) x (854 x 857)			
1	82.6	1700.0	93.8	520.0	Total Average	20.6	5.5
¾	69.3	1275.0	81.2	390.0	¾-1	32.0	10.3
½	60.9	850.0	70.7	260.0	½-¾	50.6	12.4
¼	50.4	425.0	59.5	130.0	¼-½	40.5	11.6
					0-¼	8.4	2.2
				Percent Dry Matter at Maturity 30.6			

Line 850 had a fresh weight growth period of 92.4 days during which its fresh weight increase averaged 9.1 grams per day. Its maximum rate of fresh weight increase came during the third quarter when it formed 28.6 grams of material per day. Its dry weight increase extended over 98 days or 5.6 days longer than its fresh weight increase. Its average rate of dry matter formation was 3.1 grams per day, and its highest rate was in the third quarter, 11.1 grams. At maturity 36.9 per cent of the total mass was dry matter (for purposes of these calculations, the point at which the greatest dry weight is attained has been designated as maturity). Line 853 had a fresh weight increase period of 81.2 days during which it increased at the rate of 8.1 grams per day. Its maximum rate of increase, 19.6 grams per day, was in the third quarter. The dry matter increase period extension over 91 days represented a lag of 9.8 days behind fresh weight increase. The average rate of dry matter increase was 2.5 grams per day and the maximum rate during the third quarter, 7.4. At maturity, dry matter constituted 34.5 per cent of the total weight.

The hybrid 850 × 853 showed a considerable amount of hybrid vigor. Its fresh weight increase period was 84 days, intermediate between those of the inbreds. During this period, however, the average rate of increase was 19.0 grams per day, more than double that of the faster growing inbred. The maximum rate of increase in the third quarter was 44.0 grams per day. Fresh weight at the end of the growing period was 1600 grams, nearly double that of the larger parent. Dry weight increase extended over 86.1 days or only 2.1 days longer than the fresh weight increase period. The average dry weight increase was 5.7 grams per day and the highest rate, which extended over both the second and third quarters was 15.6 grams per day. Both these figures are significantly higher than those for either inbred parent. At maturity the dry matter constituted 30 per cent of the total fresh weight. The hybrid growth factor was higher during every quarter of growth than the growth factors of either of the inbreds. The significance of this in terms of metabolic efficiency will be discussed in relation to the initial and early size differences.

Line 854 increased in fresh weight an average of 7.8 grams per day for 72.1 days to weigh a total of 560 grams at maturity. The fastest rate of increase was 20.0 grams per day, in the third quarter. The dry weight increase averaged 1.7 grams per day for 83.3 days. This was a very low dry weight increase and at maturity the dry weight of

the plants was only 145 grams, 25.8 per cent of the total weight. The fastest dry weight increase, 4.7 grams per day during the third quarter, was less than half that of most other lines. The lag between the fresh weight maximum and the dry weight maximum was 11.2 days.

Line 857 attained a fresh weight of 840 grams in 77 days, an average increase of 10.9 grams per day. This was the same total fresh weight as that of line 850 but it was attained in 15.4 days less time than in line 850. The dry weight increase of line 857 averaged 4.8 grams per day for 84 days, making a total of 400 grams, reached 7 days later than the fresh weight maximum. In the case of fresh weight, maximum growth in line 857 came in the second period during which the increase was 25.0 grams per day. Maximum dry weight increase, 13.0 grams per day, came in the third quarter.

The hybrid 854×857 was a somewhat larger plant than the 850×853 hybrid. It increased an average of 20.8 grams of fresh weight material per day for 82.0 days, making a total of 1700 grams. Its maximum rate of increase, 55.2 grams per day in the third quarter greatly exceeded the maximum rate of 850×853. Its dry weight maximum of 570 grams was reached in 91 days, 9.0 days later than the fresh weight maximum. Its greatest dry weight increase, 18.5 grams per day, came in the third quarter and exceeded significantly the greatest dry weight increase of 850×853. Both the fresh weight growth period and the dry weight growth period of the 854×857 hybrid were longer than those of the parents. The percentage of dry weight was intermediate between those of the parents at maturity, being 25.8 per cent for 854, 47.6 per cent for 857, and 33.5 per cent for the hybrid.

The double hybrid $(850 \times 853) \times (854 \times 857)$ had a maximum fresh weight of 1700 grams, the same as 854×857, and reached in approximately the same length of time, 82.6 days. Its maximum dry weight was intermediate between those of its two parents, 520 grams reached in 93.8 days, somewhat longer than the 854×857 growth period. Its maximum rate of fresh weight material formation, 50.6 grams in the third quarter, was intermediate between those of its parents and its maximum rate of dry matter formation, 12.4 grams in the third quarter, was less than that of either parent. In general, this hybrid showed somewhat more influence of the 854×857 parent than of the 850×853 parent.

Line 852 increased in fresh weight at an average rate of 13.2 grams

per day for 91.0 days, to weigh a total of 1200 grams at maturity. Both the second quarter and the third quarter had the same average rate of increase, 35.7 grams per day. Its dry weight increase averaged 3.4 grams per day for 98.0 days, making a total of 330 grams. The lag between the fresh weight and dry weight maxima was 7 days. At maturity dry weight constituted 27.5 per cent of the fresh weight.

Line 851 increased in fresh weight at an average rate of 9.3 grams per day for 81.9 days, to total 760 grams. Its greatest rate of increase, in the third quarter, was 34.1 grams per day. Its dry weight increased at an average rate of 2.4 grams per day for 99.4 days, to make a total of 235 grams. The maximum dry weight increase, 6.5 grams per day, extended over both the second and third quarters. The lag between the fresh weight maximum and the dry weight maximum was 17.5 days. At maturity the dry weight was 30.9 per cent of total fresh weight.

Heterosis is clearly evident in the growth of the hybrid 852 $\times$ 851. The average daily fresh weight increase, 32.1 grams, was more than twice that of line 852. The total fresh weight, 2700 grams, was also more than twice that of line 852. Furthermore, it was attained in 84 days, 7 days less than that required for 852, the faster growing of the parents, to reach its maximum. The fastest rate of fresh weight growth, 87.7 grams per day, extended over two quarters, as was the case in 852. The hybrid dry weight increase averaged 7.9 grams per day to total 740 grams in 93.8 days, making a lag of 9.8 days between the fresh weight maximum and the dry weight maximum. The highest rate of dry weight increase, 24.0 grams, was reached in the third quarter. At maturity dry weight made up 27.4 per cent of the fresh weight, approximately the same figure as that of 852.

Line 855 had an average fresh weight increase of 8.7 grams per day for 87.5 days, making a total of 760 grams. Its greatest rate of increase, 24.7 grams per day, came in the third quarter. Its dry weight increased at the rate of 3.3 grams per day for 98 days, to make a total dry weight of 330 grams. The fastest dry weight increase, 13.1 grams, was in the third quarter. The lag between the fresh weight maximum and the dry weight maximum was 10.5 days. At maturity, dry weight made up 43.4 per cent of the total fresh weight.

Line 856 had an average fresh weight increase of 7.8 grams per day for 79.8 days to make a total of 620 grams. Its third quarter increase rate was 24.6 grams. Its dry weight increase averaged 2.8 grams per

day for 93.1 days, making a total of 260 grams. The most rapid dry weight increase was in the third quarter, 9.3 grams per day. The lag between the attainment of maximum fresh weight and maximum dry weight was 13.3 days. At maturity, dry weight made 41.9 per cent of the total fresh weight.

The hybrid 855 × 856 shows distinct heterosis in both fresh and dry weight growth. Its average fresh weight increase was 27.3 grams per day, approximately three times that of line 855 and almost four times that of line 856. Its maximum fresh weight was 2100 grams, reached in 77 days. Its fastest growth in the third quarter averaged 83.3 grams per day. Its dry weight increased at an average rate of 8.6 grams for 84 days, to make a total of 720 grams. The lag between maximum fresh weight and maximum dry weight was only 7 days, much less than that of either parent. At maturity dry weight made up 34.3 per cent of the total fresh weight, significantly less than in either of the inbreds.

When the double hybrid (852 × 851) × (855 × 856) is compared with its hybrid parents it is seen to present a somewhat different growth picture. Its total fresh weight of 2200 grams is intermediate, but closer to 855 × 856 than to 852 × 851. But this weight is reached in 95.9 days and the average daily rate of fresh weight increase, 22.9 grams, is significantly less than that of either of the hybrid parents. In dry weight, however, the double cross is superior to the single-cross inbreds. It reaches a total dry weight of 1000 grams in 104.3 days at an average dry weight increase of 9.6 grams per day. This average is higher than the corresponding averages of either single-cross hybrid, although the highest period dry weight increase rate for the double-cross hybrid appears to be slightly less than that of 855 × 856.

The inbreds 855 and 856 occur in two other double-cross combinations (852 × 859) × (855 × 856) and (858 × 859) × (855 × 856).

Line 859 differs significantly in most growth characteristics from line 851, the other inbred with which line 852 was crossed. Line 859 had an average daily fresh weight increase of 7.7 grams, maintained over 86.1 days to make a total fresh weight of 660 grams. Its fastest increase rate was 18.1 grams in the third quarter. Its average dry weight increase was 3.0 grams, continued over 98 days to make a total dry weight of 290 grams. The most rapid dry weight increase was in the third quarter, 9.4 grams per day. The lag between fresh weight maximum and dry weight maximum was 11.9 days.

The hybrid 852 × 859 shows heterosis. Its total fresh weight was significantly less than that of the 852 × 851 hybrid, but its total dry weight was significantly more. Hybrid 852 × 859 attained a maximum fresh weight of 2000 grams in 92.4 days, and a total dry weight of 950 grams in 100.1 days, a lag of only 7.7 days behind the fresh weight maximum. Its average fresh weight increase was 21.6 grams per day, appreciably less than that of the 852 × 851 hybrid. Its average dry weight increase was 9.5 grams per day, appreciably more than that of 852 × 851. At maturity the dry weight of 852 × 859 made up 47.5 per cent of the total fresh weight, an unusually high percentage.

When 852 × 859 is crossed with 855 × 856 the resulting double hybrid shows less heterosis than either of the single-cross hybrids. Its total fresh weight was less than that of either of its hybrid parents. Its total dry weight was intermediate but closer to that of the smaller parent, 855 × 856. Both its average rate of fresh weight increase and its highest rate of fresh weight increase were less than those of either of its hybrid parents and the same holds true with respect to dry weight increase rates. The percentage of the fresh weight which the dry weight made up at maturity was intermediate between the figures for the two parents.

Line 859 was also used as a staminate parent in the cross 858 × 859. Line 858 had an average daily fresh weight increase rate of 8.1 grams, making a total of 660 grams in 81.2 days. The total mature fresh weight of 858 is thus the same as that of 859. Line 858 increased an average of 2.0 grams of dry weight per day for 88.2 days, making a total of 175 grams, which is significantly less than the total dry weight of 859. The lag between the fresh weight maximum and the dry weight maximum was only seven days. At maturity dry weight made up only 26.5 per cent of the fresh weight. Distinct heterosis is apparent in the 858 × 859 hybrid. The total fresh weight of this hybrid at maturity was 715 grams, formed at an average rate of 22.7 grams per day for 77 days, a relatively short growth period. Maximum fresh weight increase extended through both the second and third quarters. The total dry weight at maturity was 600 grams, formed at an average rate of 7.1 grams per day for 84 days. The lag between fresh weight and dry weight maxima was seven days.

When the hybrids 858 × 859 and 855 × 856 were crossed, the double hybrid approximately equalled 855 × 856 in total fresh and

dry weight but it had a much longer growth period and hence lower average rates of increase. Its fresh weight was built up over a period of 91.0 days and its dry weight over 102.2 days or 14.0 and 16.2 days longer than were required by 855 × 856 to reach its fresh weight and dry weight maxima. At maturity, dry weight made up 31.4 per cent of fresh weight, approximately the same as it did in 855 × 856.

A general comparison of the inbreds and the hybrids reveals several facts. As would be anticipated, differences among the inbreds are largely ironed out in the hybrids, and, relatively, there seems to be less difference among the double-cross hybrids than among the single-cross hybrids. In no instance does there seem to be any clear cut influence of one particular inbred line upon the growth pattern of the hybrids. This does not mean at all that in a consideration of more specific characters than general mass increase one would not find such particular influence, but in the consideration of the general characteristic of mass increase such an influence, if present, seems to be obscured by the development of heterosis in the hybrids. As a character heterosis appears to be quite independent of the initial embryo size, of the seed size, or even of the early seedling size. Neither can it be referred to the length of the growth cycle. The principal differences between the hybrids, which show heterosis, and the inbreds have to do with the attainment early in growth of a higher rate of material formation in the hybrids. Because of this the peak of growth in the hybrids is reached significantly earlier than in the inbreds. Once this peak is reached growth in the hybrids may or may not be continued over a period equal to that in the inbreds, but in terms of both fresh weight increase and dry weight increase the hybrids, in general, definitely attain maximum growth significantly earlier. Too, the maximum rate of mass increase in the hybrids is ordinarily from two to three or more times that of the maximum rate of mass increase in the inbreds. With respect to any of these factors the double-cross hybrids do not seem to have an advantage over the single-cross hybrids, although in some instances they are slightly larger plants at maturity. Advantages of double-cross hybrids would seem to depend upon factors other than those concerned with total mass. There are distinct differences among the various inbred combinations in the percentage relationship of dry weight to fresh weight at maturity, but this relationship does not seem to be associated with the development of hybrid vigor.

Discussion

These experiments were designed especially as a study of some of the problems of growth (mass increase) independent of the problems of concurrent differentiation. With the possible, but not probable, exception of certain of the lower organisms, growth over any considerable period is always accompanied by differentiation at the protoplasmic level if not at the organ, tissue, or cell level. Any comprehensive analysis of the development of an organism must take into consideration not only growth and differentiation but the interrelations of the two. Since this is so, however, it is imperative that we begin to accumulate a knowledge of general growth without breaking the characteristic down in terms which make the consideration partly one of growth and partly one of differentiation. It is unfortunate that it was not possible in the present study to include the roots and thus have a picture of total plant growth. To do so would have necessitated a reduction in the number of plants handled to the point where the data would have lost much of their significance. It is believed valid to assume that the figures on increase in shoot mass presented in the latter part of this paper will reflect in a general way total growth of the plant. Should this not be a valid assumption, they are still significant in that the results concern themselves only with comparisons among different inbreds and hybrids as to shoot growth, and in that no conclusions are drawn other than those having to do with comparative values.

The results seem to have a bearing upon several aspects of growth. In relation to many problems there has long been concern over the relationship between the size of the embryo and/or the size of the seed and the size of the plant at maturity. From species to species there obviously is no positive correlation between seed or embryo size and plant size. Within a species or among selected lines of a species the possibility of the existence of direct relationships of this sort are of importance from the standpoint of consideration of so-called quantitative characteristics.

The data indicate clearly that there is no essential correlation, among the inbreds and hybrids studied, between the size of the embryo and the plant which ultimately develops from the embryo. It has been suggested by several investigators (see Ashby, 1930, 1932) that hybrid vigor is already developed by the time the embryo matures in the seed. East (1936) contended that this is not necessarily so,

and the data in the present experiment confirm East's contention. Although sometimes hybrid embryos seem to have some advantage in size, it would seem that size of the embryo is quite unrelated to ultimate size of the plant. Neither does the amount of nutrient material stored in the seed and thus presumably available to the embryo have any direct significance for the development of hybrid vigor. From another standpoint, however, there is a relationship between hybrid vigor and seed size. Where vigorous single-cross hybrids were used as the pistillate parents, the vigor of the hybrids tended to be reflected in the size of the double-cross hybrid seed which they produced. It should be noted, however, that in general these rather uniformly large double-cross hybrid seeds produced plants no larger than, if as large as, their single-cross hybrid parents.

It is possible to detect the influence of the genetic constitutions of specific lines in certain cross combinations. Line 850 is a large embryo line. In one of the crosses in which it was used as a pistillate parent, the staminate parent line, 853, had small embryos. In this instance the hybrid had a small embryo. When 850 was crossed with line 857 which is a large-embryo type, the hybrid had large embryos. Line 852, a small-embryo type, gave small embryos with both 851 and 859, two other small embryo lines. Likewise, the influence of embryo size appears in the other single crosses. When the double crosses are considered, a level of relative uniformity in embryo size is suggested.

It would be of considerable interest to learn in what manner the genetic control of embryo size operates. The ultimate embryo size must, of course, be determined by the embryo growth rate and the length of the embryo growth period, neither of which can be presumed to be completely independent of growth of the maternal parent plant during the mid and late stages of development. The answers to questions of embryo development can come only through a different type of study. It would seem, however, that the data in this study point conclusively toward the desirability of considering growth during the early stages more carefully in terms of its physiological aspects than in terms of size progression. They also suggest, by implication at least, that growth during the embryo and seed maturation period is dominated by influences distinctly different from the dominating influences of the post-germination period. A consideration of the genetic control of development during the seed maturation period must of

necessity give attention not only to the genetic constitution of the cells of the embryo but to the genetically differently balanced endosperm tissues and to the metabolic pattern of the supporting maternal tisues. Factors of embryo-endosperm compatability are undoubtedly involved.

In the post-germination growth there are distinct general growth pattern differences between the heterotic hybrids and the inbreds. The hybrids reach a maximum growth rate earlier than do the inbreds. Presumably this indicates more rapid attainment of the optimum adjustment between the processes and reactions of the unfolding growth pattern and the environment. It is likely that the level of enzyme and other growth substance production in the hybrids is built up more rapidly. Bernstein (1943) has reported a series of analyses on amylase activity in two maize inbreds and their reciprocal hybrids. His figures indicate that the hybrids were without any advantage as to the level of amylase activity, but there definitely was an earlier mobilization of endosperm reserves in the heterotic hybrids. Bernstein has interpreted this earlier mobilization as the result of quicker germination responses rather than the cause of quicker germination responses on the part of the hybrids; however, a much more detailed analysis of the enzyme metabolism would be necessary to settle the question.

There seems to be no clear cut extension of the hybrid growth period beyond that of the inbreds, although in some instances there definitely is a less rapid falling off of the higher growth rates of the hybrids. Put simply, the hybrids complete a major part of their growth earlier, although they do not necessarily reach the end of the growth periods any earlier.

In general the average growth factors of the hybrids are two or three times as great as those of the inbreds with respect to both fresh weight increase and dry weight increase. These higher averages are the result of higher maximum rates which are sometimes characteristic of longer maximum rate periods in the hybrids than in the inbreds. Furthermore, the growth factor data also show the earlier attainment of a rapid rate of growth in the hybrids. Comparison of the growth factors for fresh weight increase and those for dry weight increase gives emphasis to the fact that the hybrid advantage lies not in such characteristics as water absorption and hence, presumably, in cell vacuolation and enlargement, but rather in a definite advantage in the rate of protoplasmic activity. Rarely is their sig-

nificantly more hybrid protoplasm than inbred protoplasm either in the embryo or in the very early seedling stages. Definitely, however, hybrid protoplasm is a more efficient "stuff" capable of reproducing itself more rapidly and in greater mass than the inbred protoplasm. This re-emphasizes the point that our consideration of growth ultimately deals primarily with the metabolic efficiency of protoplasm. Presumably, this metabolic efficiency of the protoplasm can be approached by a study of the activity of the meristematic regions in which protoplasmic activity is the dominant developmental process.

Except with reference to the matter of imposition of a ceiling upon embryo size, there do not appear in the data any clear instances of domination by single genes or small groups of genes. It must be re-emphasized that this is in a measure a consequence of the purposeful consideration of the general characteristic of growth rate rather than specific characteristics constituted of individual growth processes or perhaps of the growth of individual parts or tissues. Furthermore, the existence of marked heterosis would probably tend to obscure the influence of specific genes.

It is to be hoped that a study of specific characteristics can now be made against a background of knowledge of the general growth behavior of these inbred lines and their hybrids. If differences in differentiation can be considered in terms of these general growth differences, a knowledge of several aspects of the developmental pattern and the influences of heterosis upon the developmental pattern may emerge.

Summary

The growth of inbred and single- and double-cross maize hybrids in terms of both fresh weight increase and dry weight increase was studied during the germination period and the early post-germination period in one instance and during the grand period of growth and the maturation period in another instance. No relation was found between size of the embryo and/or size of the seed and the ultimate size of the shoot of the plants developing from the embryos.

A method was developed for the determination of the relative efficiency of the protoplasm synthesizing processes of the inbreds and the hybrids. The hybrids were found to have relatively more efficient protoplasm than the inbreds. In every case where the hybrids exhibited distinct vigor, this vigor was associated with an early attain-

ment of rates of fresh weight and dry weight increase higher than those of the inbred parents. Double-cross hybrids showed no distinct growth advantages over single-cross hybrids.

It is postulated that further studies of size differences should more profitably deal with determinations of physiological differences in the active protoplasm forming regions of the plant than with matters of size progression unless the latter may be valuable as indicators. It is further postulated that the controlling growth factors during the period of embryo and seed development differ from those which dominate in the post-germination growth stages.

References

1. Ashby, E. 1930. Studies in the inheritance of physiological characters. I. A physiological investigation of the nature of hybrid vigour in maize. *Ann. Bot.* **44**, 457-467.
2. ———. 1932. Studies in the inheritance of physiological characters. II. Further experiments upon the basis of hybrid vigour and upon the inheritance of efficiency index and respiration rate in maize. *Ann. Bot.* **46**, 1007-1032.
3. ———. 1936. Hybrid vigour in maize. *Am. Nat.* **70**, 179-181.
4. Bernstein, Leon. 1943. Hybrid vigor in corn and the mobilization of endosperm reserves. *Amer. Jour. Bot.* **30**, 801-809.
5. Blackman, V. H. 1919. The compound interest law of plant growth. *Ann. Bot.* **33**, 353-360.
6. East, E. M. 1936. Heterosis. *Genetics* **21**, 375-397.
7. Hatcher, E. S. J. 1939. Hybrid vigour in the tomato. *Nature* **143**, 523.
8. ———. 1940. Studies in the inheritance of physiological characters. V. Hybrid vigour in the tomato. Pt. III. A critical examination of the relation of embryo development to the manifestation of hybrid vigour. *Ann. Bot.* **4**, 735-764.
9. Lindstrom, E. W. 1935. Genetic experiments on hybrid vigor in maize. *Am. Nat.* **69**, 311-322.
10. Luckwill, L. C. 1937. Studies in the inheritance of physiological characters. IV. Hybrid vigour in the tomato. Pt. 2. Manifestations of hybrid vigour during the flowering period. *Ann. Bot.* **1**, 379-408.
11. ———. 1939. Observations on heterosis in Lycopersicum. *Jour. Genet.* **37**, 421-440.
12. Murdoch, H. A. 1940. Hybrid vigor in maize embryos. *Jour. Hered.* **31**, 361-363.
13. Smith, H. H. 1944. Recent studies bearing on the inheritance of quantitative characters in plants. *Bot. Rev.* **10**, 349-382.
14. Whaley, W. Gordon. 1939a and b. A developmental analysis of heterosis in Lycopersicon. *Am. Jour. Bot.* **26**, 609-616, 682-690.
15. ———, & Long, Alice. 1944. The behavior of excised roots of heterotic hybrids and their inbred parents in culture. *Bull. Torr. Bot. Club* **71**, 267-275.
16. ———. 1944. Heterosis. *Bot. Rev.* **10**, 461-498.

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A STUDY IN TRANSPLANTED RAT AND SPONTANEOUS DOG TUMORS OF GROWTH ACCELERATING AND INHIBITING FACTORS FROM DUOJEJUNAL TISSUE OF HOGS, SHEEP, AND DOGS

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The spectacular disclosures made by recent advances in nuclear physics and the interest created in the physical energies emitted by radioactive substances have, apparently, centered the attacks on fundamental cancer problems in the fields of physics and physical chemistry.

Valid theoretical considerations and animal experiments indicate that organotherapy may supply significant information concerning the autonomous growth of tumors. Many workers have chosen, empirically, substrates prepared from animal organs and tested the material for tumor growth retarding properties. Some beneficial effects have been reported.

Maisin and collaborators (12, 13, 14), developed a cancer therapy consisting of injections of brain, spleen, stomach and bone marrow, to which minute amounts of calcium, barium, and strontium were added. They reported some good results from this treatment in animal experiments and in human patients.

Blumenthal (2), and Jacobs (11), prepared mixed extracts from the duodenum, pancreas, liver, and spleen, and both reported encouraging results from this therapy in animal experiments. Some of the experimental work reported in this paper would seem to confirm certain findings of Blumenthal and Jacobs. These sources present some evidence of an observable effect upon the growth rate of tumors produced by the unphysiological concentration of protein degradation products.

More specific than these reports is the work described by Reimann

(15), in which definite functions in the growth process are attributed to certain amino acids. Sulfur, and naturally occurring compounds which contain sulfur, appear to be of great significance in growth regulatory mechanisms.

During the latter part of the nineteenth century de Rey-Pailhade (16, 17), made the interesting observation that many tissues can reduce sulfur to hydrogen sulfide. Contemporaries of this worker did not grasp the full import of his observation, but it supplied the historical background for subsequent studies of oxidation-reduction mechanisms present in plant and animal tissues.

Shortly after the turn of the century, interesting theories concerning biologic oxidations began to appear in the literature. Bach (1), and his collaborators advanced a theory for tissue oxidations and outlined what they considered to be the steps involved.

Wieland (23, 24, 25, 26, 27), in a series of articles advanced the theory that oxidation could occur in the absence of oxygen. He also outlined what he considered to be the steps involved.

Hopkins (10), and his collaborators, made a most important contribution to our knowledge of physiological oxidation; this work was done during the first quarter of the twentieth century, starting in 1921. These workers isolated from plant and animal tissues what later proved to be a tri-peptide of cysteine, glutamic acid and glycine. They called this substance glutathione. This sulfur containing compound apparently acts as an oxygen accepter and a hydrogen donor. The reactions that involve glutathione are reversible.

In 1923 the results of studies made by Warburg (19, 20, 21, 22), and his collaborators, of the carbohydrate metabolism of malignant tumors in-vitro, began to appear in the literature. These studies apparently disclosed certain metabolic properties inherent in neoplastic tissues.

During this period several investigators ascribed a special significance to sulfhydryl compounds present in protein material. Goerner and Goerner (4), noted that the growth of the Flexner-Jobling carcinoma in rats was markedly depressed by a diet deficient in cysteine.

Voegtlin *et al.* (18), demonstrated that the addition of the sulfhydryl compounds, cysteine, and glutathione caused growth to resume to a normal extent.

Hammett (5, 6, 7, 8, 9), and co-workers demonstrated by experimental procedures that sulfhydryl groups were indispensable factors

in the growth regulatory mechanisms of both normal and pathologic tissues.

Hammett also postulated that the growth stimulating and inhibiting factors were naturally occurring substances; that the natural inhibitor must be the derivative, through normal organic reaction, of the natural accelerator.

Data published by Christoffersen and Jacobs (3), reveal that the incidence of primary malignancy in the duodenum, jejunum, and ileum is very low. Hence, it would seem that tissue from this location should be good source material for the study of the growth factors in tissues, particularly the inhibiting one.

This is the first of a two part report; part one will describe the preparation of first extracts used, some animal experiments which demonstrated the presence of tumor growth modifying substances, and two methods for separating the accelerating and inhibiting factors. In part two the results obtained in additional fractionation experiments will be reported and experiments in which preparations were so modified as to change growth promoting substances into growth inhibiting substances will be outlined.

Materials and Procedures

This work was done with naturally occurring substances rather than with artifacts; therefore first extraction procedures were simple and direct. Tissue from dog, sheep, hog, and human sources was used. Hog tissue was more available, so most of the extracts were prepared from this source.

Within a few minutes after slaughter, the animal's intestines were cut an arm's length from the stomach end, washed in cold water, and transferred to cooling chambers. Laboratory preparation began with the removal of adhering fat; then the tissue was split, washed, and minced. Tissue thus prepared was divided in two portions.

One portion of the minced tissue was immersed in five and one-half volumes of 70% alcohol and a twenty-four hour extraction period followed, punctuated by occasional agitation. Following this period, the extract was then expressed through cheese cloth and filtered through paper. The clear filtrate was dried in a current of warm, filtered air at a temperature between 40 and 50 degrees Centigrade. A brown gummy concentrate was thus obtained, and became the source of preparations labeled A-FHD.

The remaining portion of minced tissue was immersed for a short period in alcohol, then spread on trays and dried in a current of warm filtered air. On the second day this material was powdered and stored in closed jars containing a few drops of chloroform. From this source the preparations labeled A-PHD were prepared.

Five grams of the gummy mass, A-FHD, was extracted with 80 ml. of normal saline, filtered, and enough saline added through the filter paper to give 100 ml. of filtrate. This was preserved with merthiolate and labeled A-FHD.

The powdered material was extracted with 70% alcohol, the extract filtered and dried to a gummy mass. Five grams of the concentrate was treated in the same manner as A-FHD.

A suitable quantity of A-PHD was dialyzed and labeled D-APHD.

In the experiments from 1934 to 1941 the sole aim was to demonstrate the presence or absence of substances that would in any way modify the growth rate of neoplasms. For this reason, no thorough statistical analysis of data was contemplated.

185 Sprague-Dawley albino rats were injected in the right hind leg with a suspension of tumor cells from a Jensen rat sarcoma. This site was chosen for two reasons; first, that growth would occur apart from the rest of the body where it might be more easily measured; second, that the rest of the body might be used as a site for injections. No injections were made directly into the tumor.

Experimental Results

When the tumors which developed in 141 rats had become hard and firm and measured at least 15 mm. in diameter, the rats were divided into four groups for the first experiment.

This experiment indicated that a tumor growth inhibiter was present in these preparations and that A-PHD was the better material for the fractionation experiment which followed.

TABLE 1
A Comparison of Results Obtained Using 3 Different Types of Tissue Extracts on Tumor Bearing Rats

Groups	Rats	Therapy	% Recoveries
1	35	A-FHD	23
2	35	A-PHD	38
3	35	D-APHD	18
4	36	Controls	0

A sufficient quantity of A-PHD was shaken with 2 volumes of aluminum hydroxide cream and centrifuged. The supernatant liquid was collected, filtered, and preserved with merthiolate. This fraction was labeled "Supernatant Liquid". The precipitate was treated with a phosphate buffer, at pH 7.4, and again centrifuged. This precipitate was discarded, and the supernatant liquid was labeled "Release". A quantity of A-PHD was dialyzed against distilled water, the dialysate collected and concentrated to a small volume and preserved with merthiolate. This was labeled "Dialysate". The bag content was filtered, preserved and labeled "Bag Content".

To test the fractions thus obtained, twenty-five tumor bearing rats were divided into five groups for the second experiment.

The results as shown in Table 2 indicated that A-PHD contained

TABLE 2
THE EFFECT OF A-PHD FRACTIONS ON TUMOR GROWTH IN RATS

Therapy	No. Days of Survival							% Recoveries
	Start No. Rats	2	5	9	12	14	18	
Supernat Liq.	5	5	5	5	4	4	0	0
Release	5	5	5	5	4	4	4	80
Dialysate	5	5	5	1	1	0	0	0
Bag Content	5	5	5	5	5	5	5	100
Controls	5	5	5	5	4	2	0	0

a growth inhibiting factor. Furthermore, it could be inferred from this experiment that this factor was composed of large molecular aggregates.

H. Wolff (28), made an analysis of protein fractions in extracts of normal tissue and malignant tumors, and characterized the proteins according to molecular size. The results seemed to indicate that there was a shift of tumor proteins toward an increase in the ratio of the fractions of lower molecular weight and size, to those of higher molecular weight and larger dimensions.

We were not successful in demonstrating growth promoting or inhibiting activity using A-PHD in five consecutive experiments. Not only did variations in temperature, pH, and aging of the preparations produce variations in results, but it seemed to be evident that individual animals would respond in a varying manner to the same type of therapy.

In a series of five experiments planned to reproduce the results

obtained in experiment 2, only preparations made from the supernatant liquid and the release were used. (There was only a small number of tumor bearing animals on hand at the time.)

Compared with the control group, use of the supernatant liquid resulted in faster growth rates in three out of the five experiments. In one experiment of this series, the growth rate was similar to that of the controls and in another, there was one regression.

With the release, in the first experiment of the series, in a group of 10 all recovered. In the second experiment, one rat from a group of 10 recovered; in the three experiments that followed, there were no recoveries and the growth rate did not vary markedly from that of the controls.

In concentrating an alcoholic extract of this tissue, from the physical appearance of a portion of the concentrate, it appeared probable that it could be dispersed throughout an oil medium. As the instability of previous preparations had been very noticeable, corn oil preparations were made in the following manner: a 5 gm. portion from the rim of the concentrate was triturated in a mortar with 25 ml. of water. Alcohol was then added gradually in sufficient quantity to give an alcoholic concentration of 95%. Later a mixture of chloroform and alcohol was used for this extraction. This was intimately mixed with two volumes of corn oil, and the alcohol and water removed at the temperature of the steam bath.

In testing the activity of this fat soluble, and heat stable fraction, 26 tumor bearing rats having unusually large tumors were used. The average diameter of these tumors was 26.6 mm. The results of this experiment are given in Table 3.

TABLE 3
THE EFFECT OF A-PHD IN OIL ON THE REGRESSION OF TUMORS IN RATS

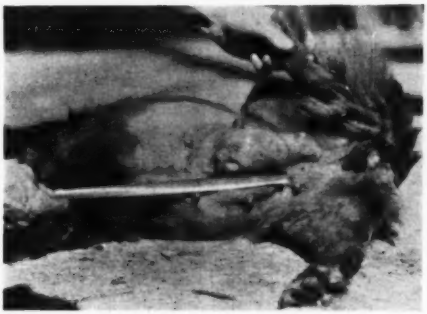
Group	Rats	Therapy	% Recoveries
1	10	Oil preparation	44.4
2	8	Controls	0

The results as shown in Table 3 indicate that the growth inhibitory factor is fat soluble and stable at the temperature of the steam bath.

In these experiments with transplanted tumors, the material was injected intramuscularly or subcutaneously. It appeared to be desirable to administer an extract intravenously in the treatment of spontaneous tumors.

A dog was secured which had developed an adenocarcinoma of the breast with metastases to the lung and liver. This diagnosis was confirmed by biopsy.

The location of the primary carcinoma is shown in Plate 1.



The dog was given a daily injection of 5 ml. of A-PHD intravenously; after 10 days the tumor mass became swollen and soft; this was followed by evidence of liquefaction. One sinus opened and discharged a creamy exudate. After 15 days the tumor mass had shrunk to about one-eighth of its original size and many sinuses had developed. The dog suddenly developed a temperature of 105 degrees F. and died. The tumor was removed and a section cut from the area marked "A" in Plate 2. A microphotograph of the slide

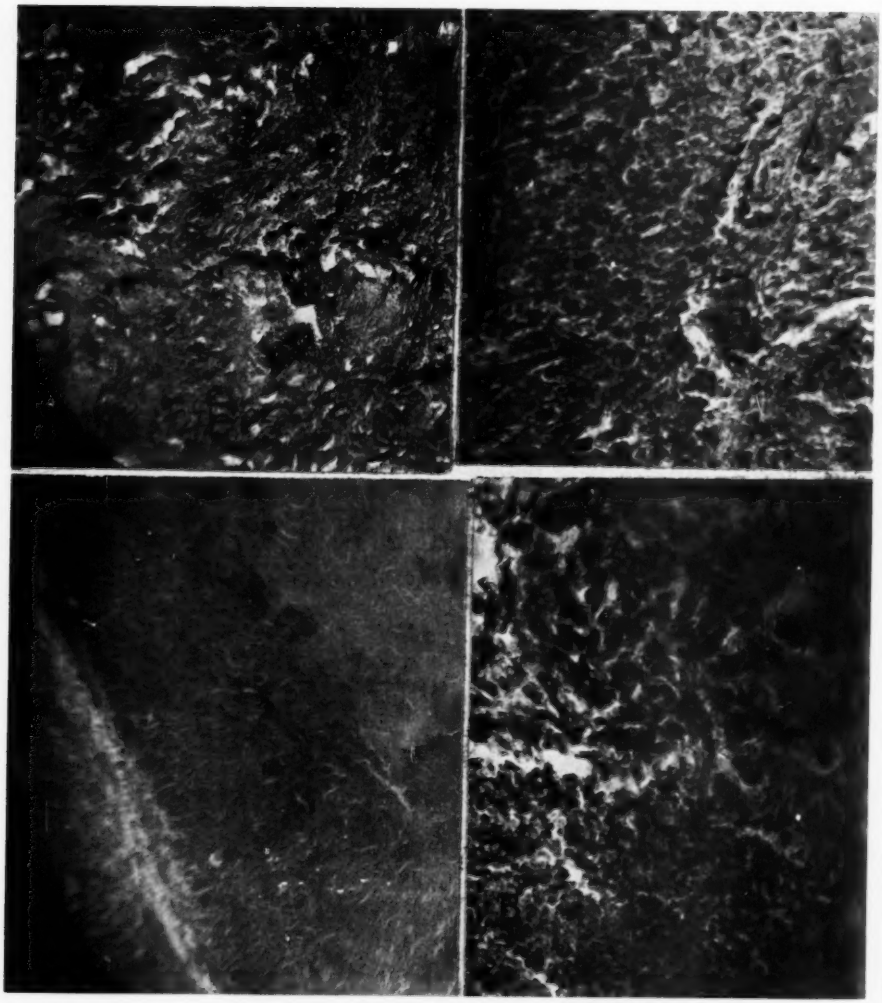


FIGURE 3.

A complete degeneration of the cells in this area; a mass of cellular debris remains.

FIGURE 4.

A microphotograph of a section from the metastasis in the lung. On the left the cancer cells are partially degenerated, and in the center only ghost cells are seen as there is almost complete degeneration. On the right the cells have completely degenerated, leaving a mass of cellular debris.

FIGURE 5.

A microphotograph of a section from the center of the metastasis in the liver. All of the cancer cells have undergone complete degeneration, leaving a granular mass of cellular debris.

FIGURE 6.

A microphotograph of another section from the metastasis in the liver. A. The cancer cells show distinct nuclei, some of which are shrunken, granular, and dark staining, denoting degeneration. B. The cells are completely broken down, and in the mass of the cellular debris may be seen many fragmented nuclear remains.

prepared from this section is shown in Plate 3. Plate 4 is a slide prepared from a section from the metastasis in the lung. Plates 5 and 6 were prepared from sections from metastasis in the liver.

The next experimental animal was a dog with a skin tumor which had apparently developed from a pigmented mole. It had several other pigmented moles, some of which showed signs of becoming malignant. The animal also had a metastasis in a regional lymph node which measured 4 cm. in diameter. This dog was treated daily with 5 ml. of an extract prepared from fresh dog duodena. After the third day the metastasis in the lymph gland and some of the pigmented moles had disappeared. Tissue paper scars remained at the original site of the moles. After ten days the tumor had liquefied, drained, and was about one-half of its original size. This was followed by toxemia and the dog died on the thirty-first day of the experiment. The body was disposed of before post-mortem was arranged.

A third dog was treated with an extract made from sheep intestines, (A-FSD). This dog had developed a carcinoma on the left shoulder with a metastasis extending from the right shoulder to the right ankle. Ten days after the treatment started the tumor mass and the metastasis became greatly swollen and on the eleventh day broke open, disclosing fluctuating masses from which drained a white creamy material. The animal died during the next twenty-four hours, apparently from toxemia.

The post-mortem revealed a white, creamy material from the area of the metastasis with no carcinomatous tissue present. The primary tumor had decreased to one-third of its original size, and when incised, it disclosed the same creamy material.

Microscopic sections from the remaining tumor tissue revealed no evidence of inflammatory reaction, but presented a picture of degenerative process. Some areas showed a rupture of cell membranes, leaving a homogenous, pink stained material, and other areas showed cells which stained poorly together with some cells with shrunken nuclei.

These preliminary experiments indicated the desirability of a more thorough study of the effect of duodenal extracts on malignant growths.

New experiments were planned and in subsequent reports descriptions will be given of additional fractionation experiments.

Discussion and Summary

In preliminary experiments with extracts of duojujenal tissue from dogs, sheep and hogs, the experimental evidence indicates that from this tissue factors may be obtained which will definitely modify the growth rate of malignant tissue. The accelerating factor appears to contain smaller molecular aggregates than those found in the inhibiting factor. Due, in part, to the lack of standardization procedures, considerable variation in the potency of the original extract was shown. Further experiments with modified preparations are deemed desirable before efforts at standardization are attempted.

References

1. Bach, A. 1913. Uber den mechanismus der oxidations-vorange. *Ber. Deut. Chem. Ges.* **46**, 3864.
2. Blumenthal, F. 1933. First International Congress of Scientific and Social Campaign against Cancer, Madrid. **1**, 743.
3. Christoferson, E. A., & Jacobs, M. D. 1939. Primary adenocarcinoma of the jejunum with perforation. *J.A.M.A.*, **112**, 1576.
4. Goerner, A., & Goerner, M. M. 1932. The Metabolism of sulfhydril compounds in tumor tissue. *Amer. J. Cancer*, **16**, 360.
5. Hammett, F. S. 1929. The chemical stimulus essential for growth by increase in cell number. *Protoplasma*, **7**, 297.
6. ———. 1930. The natural chemical equilibrium regulative of growth by increase in cell number. *Protoplasma*, **11**, 382.
7. ———. 1936. Genetics, sulfhydryl and cancer. *Science*, **83**, 57.
8. ———, & Reimann, S. P. 1940. Chemical specificity in growth and development. *Ann. Int. Med.*, **13**, 1483.
9. ———. 1941. The influence of l-proline on the growth of spontaneous tumors in mice. *Growth*, **5**, 69.
10. Hopkins, F. G. 1921. On an autooxidizable constituent of the cell. *Biochem. J.*, **15**, 286.
11. Jacobs, E. 1933. Klinische und experimentelle ergebnisse mit peroraler organtherapie bei malignen tumoren. *Ztschr. f. Krebsforsch.*, **38**, 548.
12. Maisin, J. et Vassiliadis, H. 1931. Essai d'organotherapie et de metalopterapie associes dans le traitment des cancers humains. *Compt. Rend. Soc. de Biol.*, **108**, 806.
13. ———, et Picard. 1932. Essais d'organotherapie et de metalotherapie des cancers de la peau chez l'homme. *Bull Assoc. Franc p l'etude de Cancer*, **12**, 255.
14. ———, & Vassiliadis, H. 1935. Uber die behandlung der mundholen—und hypopharynxkrebse. *Strahlentherapie*, **54**, 193.
15. Reimann, S. P. Normal intracellular constituents in relation to growth. *Cause and Growth of Cancer*: Univ. of Pa. Bicentennial Conference.
16. de Rey-Pailhade. 1888. Sur un corps d'origine organique hydrogenant le soufre a froid. *Compt. Rend. de l'Acad. de Sci. de France*, **106**, 1883.
17. ———. 1889. Nouvelles rescherches physiologiques sur la substance organique hydrogenant le soufre a froid. *Compt. Rend. de l'Acad. de Sci. de France*, **107**, 43.

18. VOEGTLIN, C., JOHNSON, J. M., & THOMPSON, J. W. 1936. Glutathione and malignant growth. *U. S. Pub. Health Rep.*, **51**, 1689.
19. WARBURG, O., & MINAMI, S. 1923. Versuche an uberlebenden carcinomgewebe. *Klinische Wchnschr.*, **2**, 776.
20. ———. 1923. Versuche an uberlebenden carcinomgewebe. *Biochem. Ztschr.*, **142**, 317.
21. ———. 1925. Uber milchsauerbildung beim wachstum. *Biochem. Ztschr.*, **160**, 307.
22. ———. 1926. Uber den stoffwechsel der tumoren. Springer, Berlin.
23. WIELAND, H. 1912. Uber hydrierung und dehydrierung. *Ber. Deut. Chem. Ges.*, **45**, 484.
24. ———. 1912. Zurbrennung des kohlenoxyds. *Ber. Deut. Chem. Ges.*, **45**, 679.
25. ———. 1912. Studien uber den mechanismus der oxydationsvogrange. *Ber. Deut. Chem. Ges.*, **45**, 2606.
26. ———. 1913. Uber den mechanismus de oxydationsvorgange. *Ber. Deut. Chem. Ges.*, **46**, 3327.
27. ———. 1914. Uber den mechanismus der oxydationsvorgange (111). *Ber. Deut. Chem. Ges.*, **47**, 2805.
28. WOLFF, H. 1905. Ein beitrag zur chemi des carcinoms. I. Mitteilung. *Ztschr. f. Krebsforsch.*, **3**, 95.

Growth, 1950, **14**, 167-185

ISOLATION OF FUNGI FROM TRANSPLANTED, CHEMICALLY-INDUCED AND SPONTANEOUS TUMORS

II. Cultural and Microscopic Observations, with Particular Reference to Sarcoma 37 and Mammary Carcinoma of Mice

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The isolation of fungi from tumor tissues has been reported by the author (Diller, 9 and 10) recently, and by many other investigators at the turn of the century as indicated in the earlier paper. During the past decade a number of references have appeared dealing with the isolation of *Blastomycetes* from human lymphosarcoma and Hodgkin's nodes (Cawley, 5; Curtis, 8; Cohen, 7; and Da Silva, 9). From our studies of mouse tumors it was first concluded that the fungi isolated were specific for particular types of tumor, but further study suggests that one group of organisms (*Blastomycetes* or allied forms) are common to all types of mouse tumor and to those human tumors, which we have thus far studied; and that, in addition, in the case of mouse tumors, there may be secondary fungus infections that are either constant for certain mouse strains or for certain mouse tumors.

For example, from tumors of "A" strain mice we consistently isolate an as-yet-unidentified imperfect fungus which produces a pinkish-brown pigment, in addition to the yeast-like form which is similar to that isolated from all the other tumors. From mammary carcinomas of inbred C3H mice carrying the "milk-factor virus" we have isolated regularly a common fungus, *Alternaria,* (Figs. 4, 16) in addition to the non-sporulating form. *Alternaria* is found in the intestinal wall of C3H nurslings, in the mammary tissue prior to appearance of tumors, and in the mammary carcinoma which arises spontaneously in females of this strain. This same form (Fig. 19) has been isolated

from methylcholanthrene-induced tumors produced in the C3H strain. Some of these chemically-induced tumors yield also the non-sporulating fungus, *Geotrichum*. A comparison of the cultures from several strains of mice appears in Plate I, Figs. 1 to 7. Figures 8 to 11 show some of these at high magnification as demonstrated in Cotton Blue preparations.

Two different fungi have been consistently isolated from sarcoma 37. The infection is not limited to tumors grown in our laboratory, since we have been able to trace the organisms in microscopic preparations of sarcoma 37 from various sources made over many years. One such set of slides was prepared twenty years ago at the University of Pennsylvania from tumors originating in the Crocker Institute. These slides were paraffin preparations stained in iron-hematoxylin, in which the fungal structures were not recognized. Recently, the application of other stains to smears of tumor tissue has made possible such identifications but, once recognized, the fungi can be located in less favorable preparations.

The dominant form which appears when sarcoma 37 tissue is streaked on Sabouraud's agar is a Zygomycete, *Syncephalastrum racemosum*. It is characterized by a non-septate branching mycelium (Fig. 8). The tips of the sporangiophores are swollen into large fat-filled vesicles from which catenate sporangia radiate (Fig. 12). In smear preparations the sporangiophores are almost invariably dissociated by mechanical operations, but sporangial vesicles remain intact, and these are demonstrable in acetic orcein preparations (Figs. 13, 14 and 15). They are filled with fat droplets and may contain a single oval nucleus.

The existence in sarcoma 37 of the unidentified form of imperfect fungus probably complicates our microscopic interpretations considerably, and some of the structures which we observe in tissue may belong to the imperfect fungus and not to *Syncephalastrum*, since it is not impossible that spore stages may occur in tissue even though the fungus does not sporulate *in vitro*.

When sarcoma 37 tissue is streaked on Sabouraud's agar, *Syncephalastrum* overgrows the plate very quickly, but on Littman's ox-gall agar it is possible to separate the colonies of the imperfect fungus from the Phycomycete. Current antigenic experiments in collaboration with Dr. D. R. A. Wharton of this Institute have indicated that when *Syncephalastrum* is suppressed, the imperfect form grows

abundantly when tissue of the antigenically-treated tumor is streaked on culture medium. The potential antagonism of these two organisms may play a significant part in the growth rate and spontaneous regression of the transplanted tumor. If further experimentation bears out these preliminary findings, there may be some hope of using one or the other of the fungi as an antibiotic.

When we injected suspensions of *Syncephalastrum* spores into the peritoneum of the mouse, the new growth elicited showed gradual transformation from a mycelial form into conidial stages (which are the ones usually encountered in tumor tissue) as the mass proliferated outward from the implant. Although it is now possible for us to interpret these structures in routine histological preparations, they are much more clearly demonstrated by means of specialized techniques such as Benda-Kull, Pianese, Protargol, and Robinow, and particularly by the new McManus technique (17) for demonstration of carbohydrates.

No sexual stages have been observed on agar cultures nor have they been recognized in microscopic preparations of tumor tissue with the possible exception of hanging drop cultures grown in extract of mouse brain and stained with Benda-Kull. This technique is particularly useful for demonstration of mycelium since osmic acid impregnates fatty substances, which may comprise as much as 70% of fungal mycelium.

There could be no doubt but that the organisms isolated from sarcoma 37 are parasitic since their presence in the cells results in widespread destruction. In an earlier paper (11) we noted that unattenuated cultures of fungi freshly isolated from tumor tissue were highly pathogenic to mice. When heavy suspensions of spores were injected into the peritoneal cavity of either Swiss or C3H mice, there resulted in every case a number of scattered nodules—most of them attached to the outer surface of the intestine. The new growths, cut in paraffin and stained with hemalum and eosin were presented as unknowns to a competent pathologist who diagnosed them as "possible neoplasms, though the presence of numerous macrophages suggests rather that they are foreign body reactions." Similar, though larger, masses of tissue produced by injection into the leg muscle, were recoverable in 3 to 4 weeks. No scattering of the fungus could be detected in other tissues at autopsy. Because of the ability of the mouse to resorb these lesions in a rather large proportion of the

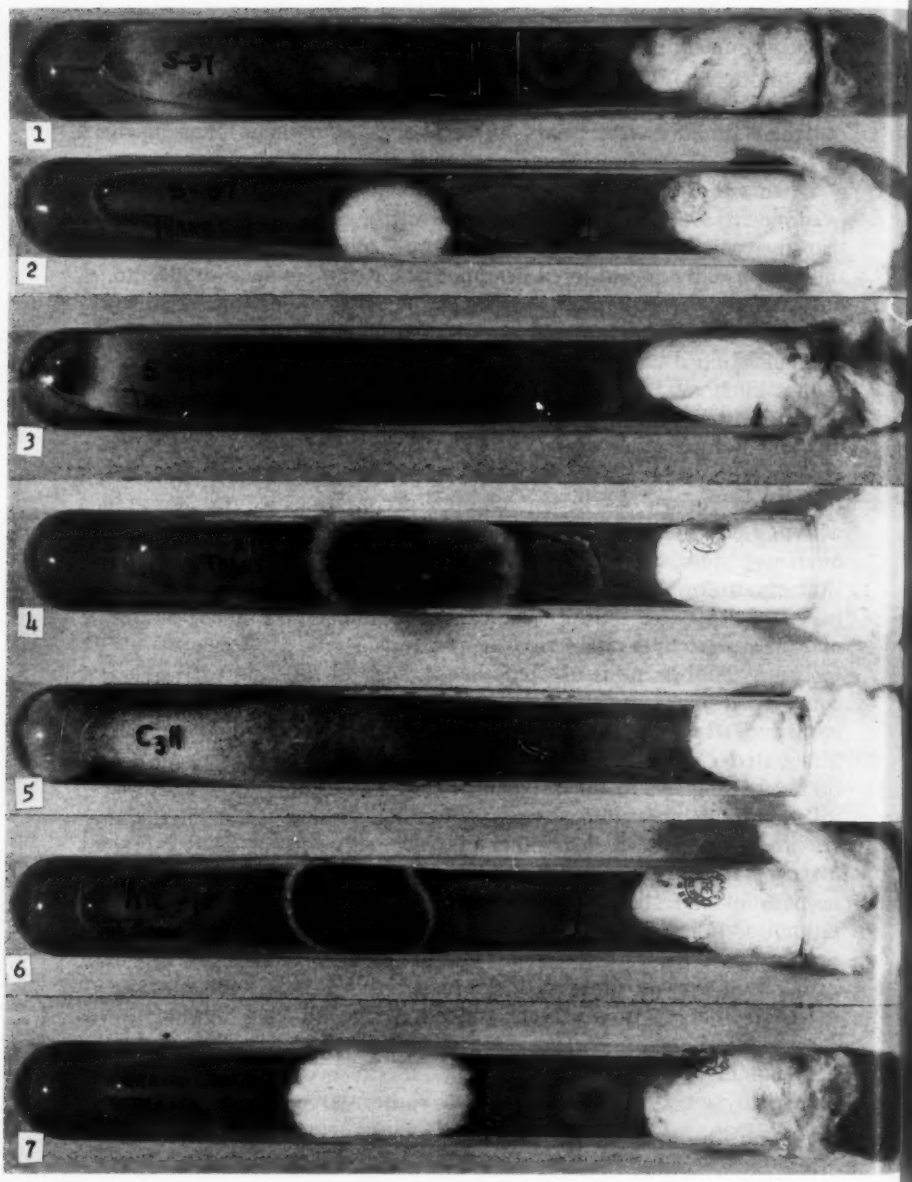
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PLATE I.

Figures 1-7. Agar slant cultures of fungi isolated from various tumors..

Fig. 1. *Syncephalastrum racemosum* from Sarcoma 37.
2. Unidentified imperfect fungus from Sarcoma 37.
3. *Syncephalastrum racemosum* from Sarcoma 180.
4. *Alternaria* from C3H mammary carcinoma.
5. *Syncephalastrum racemosum* from the same tumor as Fig. 4.
6. *Alternaria* from methylcholanthrene-induced tumor in C3H stock.
7. Imperfect fungus from a pyogenic granuloma (human cervical lymph node).

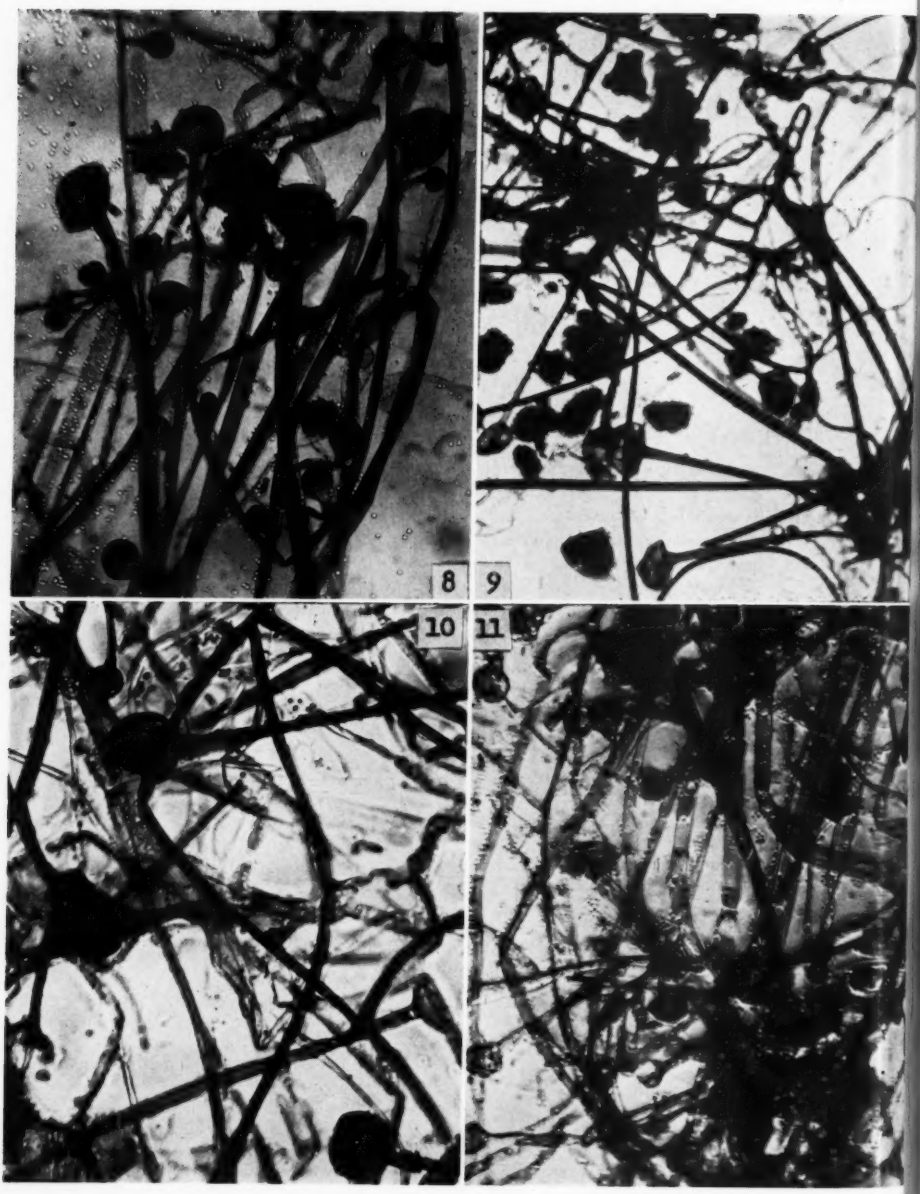
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PLATE II.

Fig. 8. Cotton Blue preparation of a culture of *Syncephalastrum* isolated from Sarcoma 37. X 150.

9. *Syncephalastrum racemosum* (Cotton Blue preparation of culture freshly isolated from primary mammary carcinoma of C3H mice). X 150.

10. Unidentified Phycomycete from human breast carcinoma. Cotton Blue preparation of living culture. X 150.

11. *Syncephalastrum racemosum* freshly isolated from Sarcoma 180. Cotton Blue preparation. X 150.

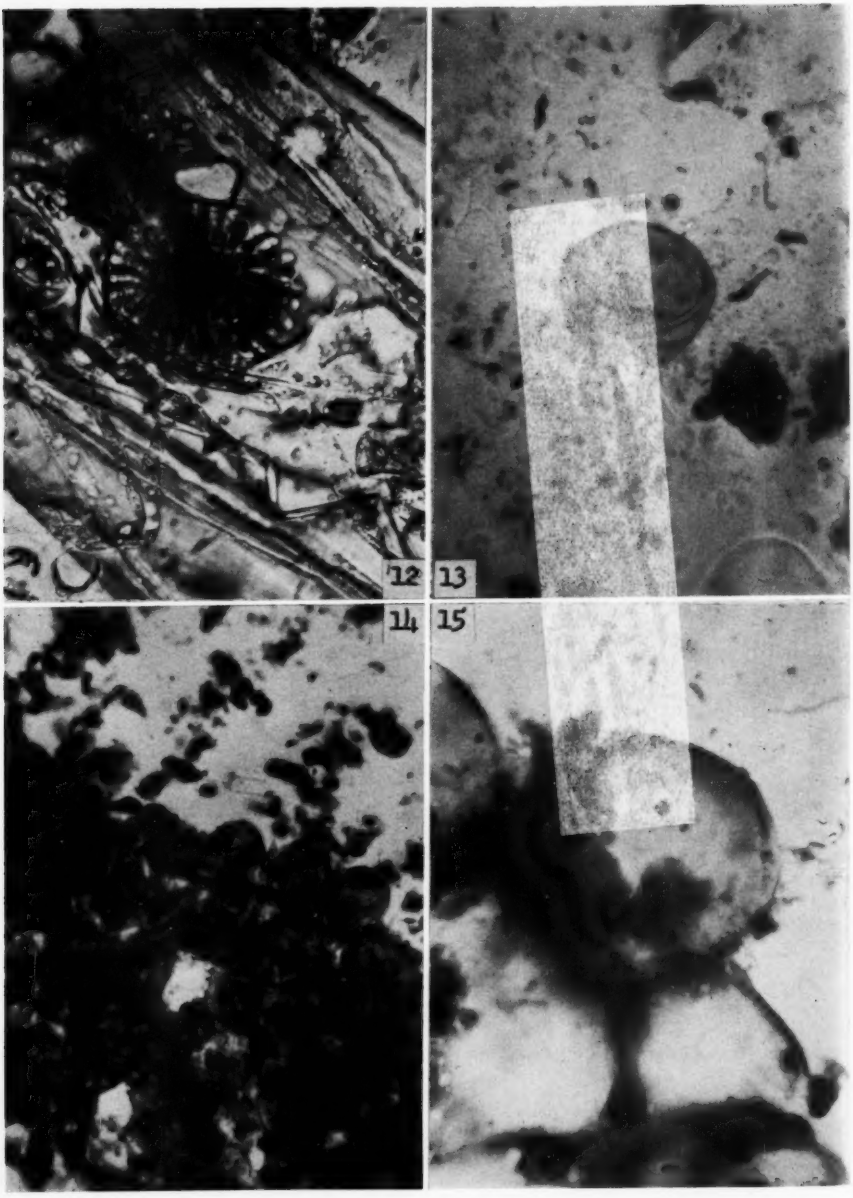
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PLATE III.

Fig. 12. Fruiting body of *Syncephalastrum* (Cotton Blue preparation of living culture) freshly isolated from Sarcoma 37. X 150.

13. Central portion of sporangial vesicle after spores have dropped off, showing an empty sac with one nucleus and some fat droplets, faintly stained by acetic orcein, found in smear preparation of tissue of Sarcoma 37. X 900.

14. Teased mass of tissue from Sarcoma 37, smeared in acetic orcein. Masses of sporangial vesicles, similar to the one shown in figure 13, at periphery of tissue. X 440.

15. The same at higher magnification. X 1200.

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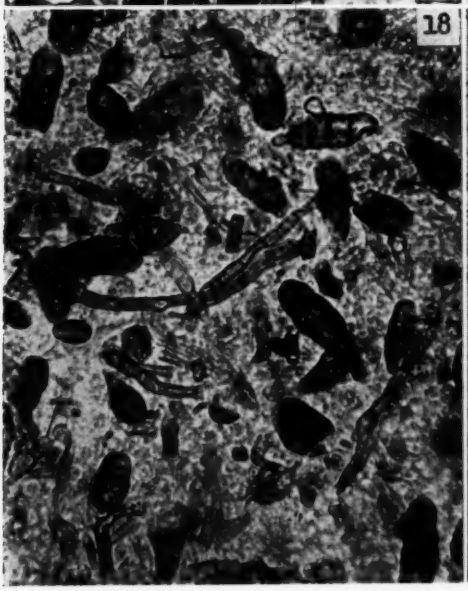
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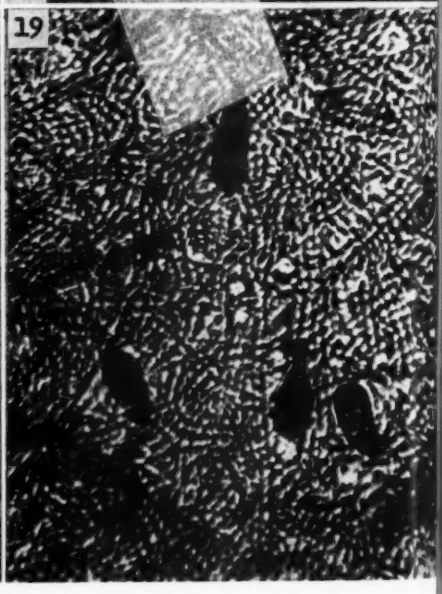
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PLATE IV.

Fig. 16. *Alternaria* isolated on Sabouraud's agar from mammary carcinoma of C3H mouse, stained in Cotton Blue. X 440.

17. Small nodule induced in peritoneum of hybrid white mouse by injection of spore suspension of *Alternaria* isolated from C3H spontaneous mammary tumor. X 150.

18. Central portion of the nodule described in figure 17. Paraffin section stained in hemalum and eosin. X 440.

19. Cotton Blue preparation of living culture of *Alternaria* isolated from a fibrosarcoma induced in C3H strain mouse by injection of methylcholanthrene. X 440.

cases, it was thought that possibly a higher percentage of permanent "takes" might be obtained by using suspensions of cultures freshly isolated from tumor tissue. For this experiment we prepared a luxuriant culture of *Syncephalastrum* which had sporulated freely 48 hours after the tissue was streaked on Sabouraud's agar. A thick suspension of spores in .05 cc. of saline solution was injected by trocar, both intramuscularly and intraperitoneally, into 6-week old C3H and Swiss mice. Two days after injection some of the C3H mice died, and all were dead on the fourth day, regardless of the route of injection. Swiss mice also died on the second day and all were dead on the sixth day post-injection. When the experiment was repeated, neither Swiss nor C3H mice survived beyond the fourth day. All normal tissues, except blood, of these mice, taken just before death and plated on Sabourad's agar, yielded abundant cultures of *Syncephalastrum* and the spleens were shrunken to about one-fifth normal size. Similar results were obtained after injecting *Alternaria* spores into C3H and Swiss mice. Apparently these fungi are highly pathogenic to mice when first isolated from tumor tissue, but become attenuated in sub-culture. No data are at present available as to the exact number of generations of sub-culture required to produce maximum new growth with optimum survival.

Attempts to produce new tumors by the introduction of fungal isolates may or may not cast some light on possible etiology. Failure to elicit a growth which satisfies the pathologist's criterion of a neoplasm would not necessarily be conclusive. The life history of these organisms may be very complex, and the necessity of being introduced into certain tissues at certain stages of the life history of either parasite or host, or even of passing through an intermediary host, is not beyond the realm of possibility.

Discussion

It is possible that the presence of fungi in tumor tissue creates a toxic state which may be responsible for some of the spontaneous necrosis encountered in our sarcoma 37 implants. Also, the spontaneous resorption of implant tissue may be related in some way to the immune reactions of the host to its contained parasites. Henrici (14) notes that one of the outstanding responses of host tissues to the presence of fungus infection is hypertrophy and hyperplasia of the tissues, and increased mitotic activity. Although ungoverned cell

division is commonly assumed to be the outstanding feature of neoplastic disease, we have for several years been puzzled by the fact that many tumors do not, as a matter of fact, exhibit undue mitotic activity. In a detailed study of early implants of sarcoma 37 we noted (unpublished data) that marked mitotic activity occurs three days after implantation at a time when capillary connections have just been established. Polyploid nuclei and multipolar spindles are common. As the tumor grows older, mitotic figures appear to be encountered less and less frequently, although without special studies of the mitotic rates this is hard to evaluate from fixed preparations, since it is known that such activity in tumors occurs in .waves. Examination of smear preparations of tumor tissue, however, shows that the fungus cells continue to reproduce by internal septation or by budding and at least a portion of the tissue in older sarcoma 37 implants may be of plant origin.

That the existence of these fungi in transplanted tumors may be merely the perpetuation of a long-standing infection is entirely possible, but this can hardly be the case when we are dealing with spontaneous tumors, or even chemically-induced ones from which, as heretofore mentioned, we have isolated similar or identical forms. In any event, steps should be taken to rid our experimental material of these contaminants; otherwise, the value of the results of experimentation with these tumors is greatly impaired, not only from the point of view of microscopic interpretation, but for any type of chemical measurement. At present, we may be dealing simply with a mélange of plant structures, spores, and mammalian tissues.

As has been noted elsewhere, the isolation of fungi from tumor tissue has been made by other investigators over and over again, and most of the earlier workers believed that they had found the causative organism. However, the accounts, e.g. that of Clarke (6), were often so confused and the results of attempting to produce new tumors so inconclusive, that the theory of parasitic etiology fell into disrepute. Of early workers who claimed successful reinoculation, Sanfelice's account (23) is most credible. His method was to re-implant the granulomas obtained on injection of fungal filtrates, whereby he claimed he had produced neoplasms identical with malignant growths of humans. The diagnosis of these growths was confirmed by such noted pathologists as Metchnikoff (18 and 19).

The presence of bodies which did not appear to be normal cyto-

plasmic constituents in cancer cells was noted as early as 1855 by Virchow (25) who described in malignant cells, double-contoured objects with central nuclei containing "coccus-like" granules. These bodies divided spontaneously. He believed that they were endogenous cells arising within the malignant ones, and not parasites. Russell (22) in 1890 described spherical "fuchsine bodies" which were found singly, or in clusters, in cells of cancerous tissues which he identified as spores of a yeast-like organism. Soudekewitch (24) found both intracytoplasmic and intranuclear inclusions in 59 different carcinomas. His figures drawn from preparations fixed in osmic acid are very similar to those we have observed in Benda-Kull preparations. Metchnikoff (18 and 19) examined the material and believed that the bodies were true parasites and not cell products. Burns and Schenken (4) studied intracytoplasmic inclusion bodies in cells of spontaneous primary hepatomas of mice. These showed a doubly refractile rim, granular contents, and single or multiple pale-staining bodies which suggest yeast-like organisms. However, they were unable to isolate these bodies on culture medium. Some of the intracytoplasmic structures in cells of sarcoma 37, as demonstrated by different techniques, appear in Figs. 21-23. Whether these bodies enter the cells autonomously, or are phagocytised by the tumor cells, is unclear. References to many others who described parasitic cell inclusions will be found in an earlier paper (Diller, 11).

Alexieff (1) found in cancer cells a parasite to which he gave a new name, *Chlamydozoon perniciosum*, and which he believed was "in reality a fungus." Clarke (6) described and figured structures very similar to those which we have observed in tissues of human mammary carcinomas and came to the conclusion that the conditions described were due to the presence of a fungus.

Many of the earlier investigators were able to cultivate organisms *in vitro*. To cite but a few examples: Bra (2) found in 204 neoplasms, both sarcomas and carcinomas, from all parts of the body, a fungus which he thought was "probably an Ascomycete." Grown on acidified gelatin the fungus produced ruby-red spores about one micron in diameter which recall the type of culture obtained by us from lymph nodes of Hodgkin's patients. Braithwaite (3) cultured one hundred and fifty different tumors and studied the spores and mycelium in unstained preparations. "In not a single primary malignant growth was the fungus absent." The work of Sanfelice has already been

mentioned. He isolated *Saccaromyces neoformans* from carcinoma of the human kidney and inoculated them into cats and dogs. This produced neoplasms "which were identical to those of man" and which were pathogenic not only to mammals but to birds.

Since the publication of our preliminary abstract (Diller, 10), the work of a number of contemporary investigators has been called to my attention. Wuerthele-Caspé (26) has demonstrated in many types of tumor tissue, including the Rous sarcoma, and other supposed virus tumors of animals, micro-organisms which are acid fast at some stage in their life history. For this reason she believes these to be Mycobacteria. However, the appearance of the organisms in culture medium and in yolk sac cultures is almost identical to that of organisms found by Gerlach (12) in 1,019 tumors of man and animals. He considers these organisms, which are capable of passing through bacterial filters, to be minute fungi. He calls them *"Micromyces blastogenes"* following the terminology of Mori (20 and 21). According to Gerlach, the organisms behave like filterable viruses when cultured in the yolk sac of the chick. He further reports that he has obtained true tumors in 4% of the cases studied when the organisms are re-injected into experimental animals, following yolk sac culture. Microbiologists whom I have consulted offer the possible criticism that the fungi studied by Gerlach may simply be vectors for a virus. Since the tumors studied by Gerlach include many types that are not known to have a virus origin, such a finding, if substantiated, would be extremely significant.

Every effort is being made in our laboratory to reconcile the results of these investigators with our own through the application of comparative techniques. It is possible that we are all working with a group of organisms which are demonstrable at different levels by means of different techniques; or that they are co-existent in some type of symbiosis. Chemical studies will be made concomitantly in an effort to determine whether the fungi produce carcinogenic substances that could conceivably prepare the way for invasion of tissues by still smaller organisms, of which they may be merely the vectors. From personal correspondence with Leyton, who has worked out a method of demonstrating mycelium in tissues (16), it appears that the fungi which she isolated from human tumors are regarded by her colleagues as probable secondary vectors of viruses. "However," she writes, "it has been established that any tiny atom of the proto-

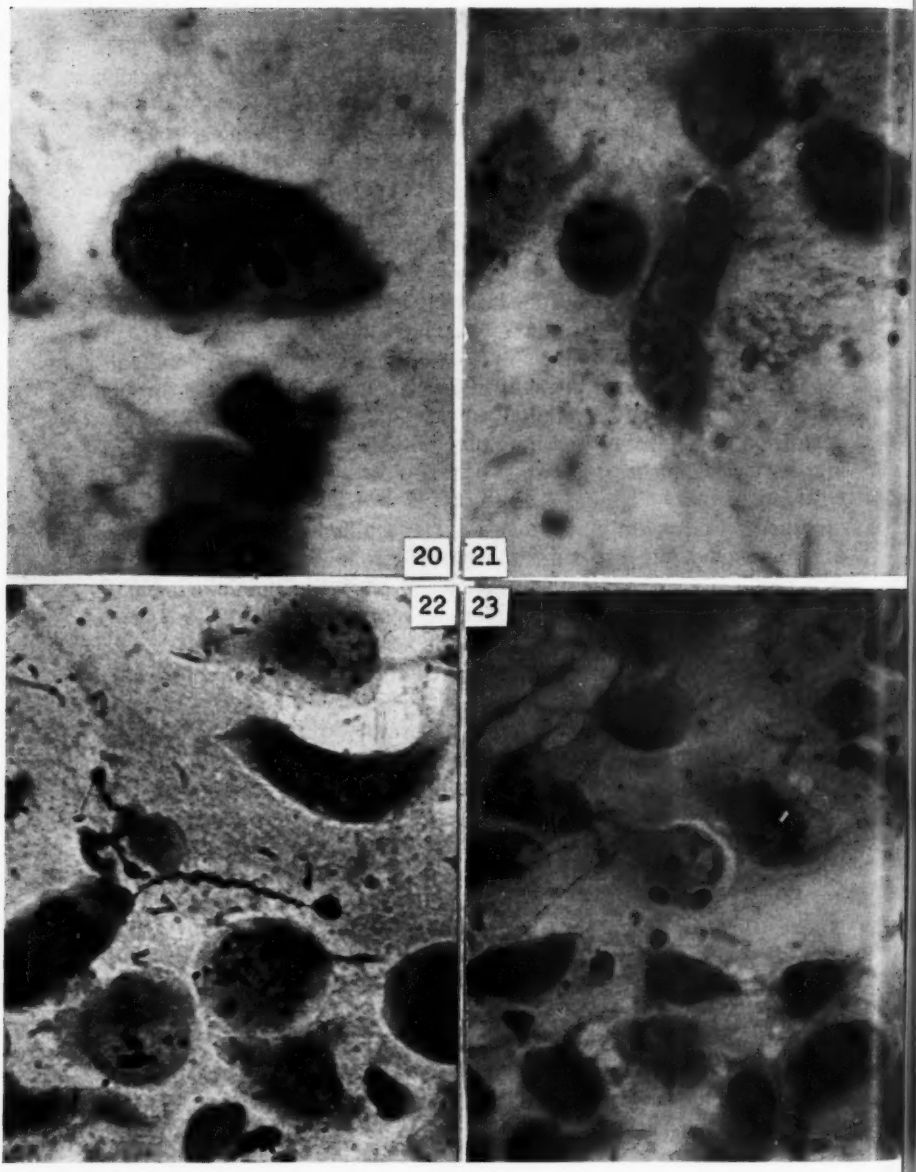
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PLATE V.

Fig. 20. Single cell of Sarcoma 37 from a smear preparation stained in Robinow. Note inclusion bodies. X 900.

21. Single cell of Sarcoma 37 from a smear preparation stained with Protargol. Spore case and escaped spore enclosed (or engulfed) by the cytoplasm.

22. Smear preparation of Sarcoma 37 prepared by the Benda-Kull method. Coiled thread appears to be a hypha attached to a sporangium leading into, or away from, a spore (at tip of arrow) inside the cytoplasm of a sarcoma cell. X 440.

23. Same type of preparation as figure 22. Three inclusion bodies in Sarcoma 37 cell, observable at one optical level; a fourth is obscured and was observable only on focusing the fine adjustment. X 440.

plasm of a fungus can reproduce the whole cycle of mycelium, spore, swimming form, etc. and that the so-called viruses might well be but atoms of fungal threads." This is a theory similar to that of Glover (13), which has never been disproved, even though others who have attempted to reproduce his results, e.g. Kolmer (15) have been unable to do so using Glover's medium. However, from some of the neoplasms tested, Kolmer isolated micro-organisms (not identified) which were different from those described by Glover.

Whatever the significance of these micro-organisms in relationship to tumor growth, their frequent, if not universal, occurrence must be taken into account, not only in the evaluation of cellular relationships, but because of the effect of their metabolic activities on the chemical constitution of the tumor tissues. With the new techniques and the new optical aids now available to us, it should be possible to follow some of these interesting observations and theories to a more definite conclusion.

Summary

The isolation of fungi from transplanted, induced and spontaneous tumors of the mouse and from human tumors and the microscopic appearance of some of the fungal structures in tumor tissue were reported in an earlier paper. The cultural characteristics and appearance of the forms isolated from several mouse tumors are described in the present paper. These organisms, freshly isolated from tumor tissue, were found to be highly pathogenic to mice when spore suspensions were re-injected either intra-peritoneally or intra-muscularly. The effects are apparently attenuated during subculture, and after several transfers through slant cultures, the injection of the organisms caused no deaths and induced new growths in mice, though these appeared to be granulomas and not true tumors.

Recent reports on the isolation of micro-organisms from tumor tissue by other investigators are discussed in detail.

References

1. Alexieff, A. Sur un Chlamydozoaire parasite des Protozoaires. Sur le Chlamydozoaire du cancer. *Arch. de Zool. exp. et gen. 5th ser.* **5**. Notes et revue *ci*, 1912-1913.
2. Bra, M. 1898. D'un champignon parasite du cancer. *C. R. Soc. Biol.* **5**, 1050-53.
3. Braithwaite, J. 1895. On the micro-organism of cancer. *Lancet* **1**, 1636-38.
4. Burns, E. L., & Schenken, J. R. 1943. Spontaneous primary hepatomas in mice of strain C3H. IV. A study of intracytoplasmic inclusion bodies and mitochondria. *Cancer Res.* **3**, 697-701.
5. Cawley, E. P., & Curtis, A. C. 1948. Histoplasmosis and lymphoblastoma. Are these diseases related? *J. Invest. Dermat.* **11**, 443.
6. Clarke, J. J. 1922. Protists and disease. Vol. 6.
7. Cohen, M. 1944. Binocular papilledema in case of torulosis associated with Hodgkin's disease. *Arch. Ophthalmol.* **32**, 477.
8. Curtis, F. 1896. Contribution a l'étude de la saccharomycose humaine. *Ann. de l'Inst. Pasteur.* **10**, 449-68.
9. Da Silva Lacaz, C., et al. 1948. Blastomicose Sul-Americana associada a molestia de Hodgkin. *Cancer Current Lit.* **2**, 574.
10. Diller, I. C. 1949. Fungi associated with tumor tissues. *Anat. Rec.* **105**, 24.
11. ————. Isolation of fungi from transplanted, chemically-induced and spontaneous tumors. I. General considerations. Cancer Research (in press).
12. Gerlach, F. 1948. Krebs und obligater Pilzparasitismus. Urban & Schwarzenberg. Wien. 211 pages.
13. Glover, T. J. 1926. Bacteriology of cancer. *The Canada Lancet and Practitioner* **67**, 5.
14. Henrici, A. T. 1940. Characteristics of fungus diseases. *J. Bact.* **39**, 113.
15. Kolmer, J. A., Harkins, M. A., & Soleeby, E. 1937. An ineffectual attempt to cultivate the Glover organism from carcinomas. *J.A.M.A.* **89**, 1868.
16. Leyton, H. G., & Leyton, G. B. An account of some of the more recent scientific investigations which gave rise to the treatment of cancer with allyl isothiocyanate. Med. Times, London, April, 1939. 6 pages.
17. McManus, J. F. A. 1948. Histological and Histochemical Uses of Periodic Acid. Stain Technology, **23**, 3. 99-108.
18. Metchnikoff, E. 1890. Remarks on carcinomata and coccidia. *Brit. Med. J.*
19. ————. 1892. Note au sujet du memoire de M. Soudekewitch. *An. de l'Inst. Pasteur.* **6**, 158-159.
20. Mori, N. 1925. Sulla Etiologia del Cancro. Morgnani, No. 24. Pp. 1-14.
21. ————. 1949. Filtrierbare Vira und Krebs im Einklang mit meiner Hypothese über deren myzeliale Natur der Krebsarzt. Urban & Schwarzenberg. Wien.
22. Russell, W. 1890. A characteristic organism of cancer. *Brit. Med. J.* **2**, 1356.
23. Sanfelice, F. 1898. Ein weiterer Beitrag zur Aetiologie der bösartigen Geschwulste. *Centralb. f. Bakt. u. Parasitenk.* **24**, 155-58.
24. Soudekewitch, J. 1892. Recherches sur le parasitisme intracellulaire et intranucleaire chez l'homme. *Ann. de l'Inst. Pasteur.* **6**, 145-57.
25. Virchow, R. 1892. Bemerkungen uber die Carcinom-zellen-Einschlusse. *Virchow's Arch.* **127**, 188.
26. Wuerthele-Caspé, V. 1948. Mycobacterial forms in tumor tissue. *N. Y. Micr. Soc. Bull.* **2**, 215-18; 19-26.

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BOOK REVIEW

Biologie der Goethezeit. Adolf Meyer-Abich. Hippokrates-Verlag Marquardt & Cie, Stuttgart, The Chronica Botanica Co., Waltham, Mass. 1949. $5.50.

REVIEWED BY PHILIP R. WHITE

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The title of this book, "Biology of the time of Goethe" is certainly a misnomer. "Goethe's Biology" or "Biology of Goethe and his Circle" would have been more correct.

Dr. Meyer-Abich starts out with the premise that Goethe was strictly a neo-Classicist, an Aristotelian in his science rather than a "Modern" in the sense of the post-Renaissance. Whether Abich realizes it or not the result is to characterize Goethe as *not* belonging in his time. I must say that he makes out a very good case for this point of view. The quotations from Goethe which make up most of the book, and from his closest friends: Forster, von Humboldt, Oken, Carus, von Baer, and Müller show a man who although extraordinarily keen and alert in his observations, encyclopedic in the breadth of his attentions, profound in certain types of analysis, is nevertheless totally blind to what the modern world (and that includes everything since the Renaissance) would consider the significance of his facts in the "World of Reality." Nowhere is this more marked than in his consideration of plants, and this is of course of especial interest to me. Is he interested in the uses of plants to man as were Fuchs and Brunfels, in the behavior of plants as living creatures as were Hales and Knight and Köhlreuter, in the ordering of plants in God's scheme of things as were Linnaeus and de Candolle? No, these aspects of biology escaped him completely and he went about from Sicily to the Northlands searching for the "Urpflanze." That is a concept so foreign to modern thought that I fear most modern readers will misinterpret it without explanation. Goethe does not mean, by Urpflanze, the "original plant" from which all others have been derived in the Darwinian sense. He meant rather that plant which so nearly as possible epitomizes those traits and

characters which inhere in the idea PLANT. Aristotelian that is, with a vengeance, and so completely so that Theophrastus himself, who was Aristotle's outstanding student, would scarcely have understood it! Goethe was not interested in the plan of the Universe but only in grasping the *Idea* nascent in God's mind when first the dream of a Universe took form! In other words, Goethe identifies himself with the Godhead, nothing less, and has no interest whatever in Man, the Earth nor Plants and Animals as they fit into Man's world.

I cannot agree with the author that, "Goethe, the Natural Scientist would be counted one of the greatest spirits in his field if Goethe, the Poet, had never existed." (p. 37). Scientist he was only in his own imagination and that of his philosopher admirers. In all of the quotations which Meyer-Abich chooses to illustrate Goethe's "Science" he remains first, foremost and always a Philosopher-Poet, *not* a Scientist. Perhaps that is why Goethe's theory of the nature of color, of which he was so proud that he dared to belittle Newton, has already in 100 years been buried and forgotten. Goethe's science is remembered chiefly for his speculations on the nature of fossils and for his introducting into our language the word, "morphology."

Pride goeth before a fall and no amount of quotations chosen by another philosopher will serve to place even one of the greatest poets of all time on a pedestal which is not rightfully his. The biology of Goethe's time was that of Hales and Knight, Lavoisier and Malpighi, Köhlreuter and Linneus, Schleiden and von Mohl, not of Goethe and Carus and Müller.

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THE EFFECT OF MILK SUPPLEMENTS ON THE GROWTH OF CHILDREN WITH NUTRITIVE FAILURE: II. HEIGHT AND WEIGHT CHANGES*

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In a previous report, Spies and Dreizen (1) summarized the changes which appeared in the Wetzel Grid patterns of 82 chronically malnourished children, 41 of whom received supplements of reliquefied milk solids. The criteria for the selection of the children, the nature and manner of administration of the supplements, the principles of therapy, and the methods of study were discussed in detail in this report. All of the subjects were white, of Anglo-Saxon extraction and for the most part natives of north-central Alabama. They ranged in age from four to fifteen years. The 82 children were paired on the basis of (a) sex, (b) comparative roentgenograms of the left hand, wrist and forearm, (c) general body type, and (d) apparent prevailing phase of growth. One child in each pair received as a supplement the equivalent of one quart of either whole or nonfat milk six days a week for a period of twenty months. Twenty-two children (9 boys and 13 girls) received nonfat milk and 19 children (9 boys and 10 girls) received whole milk.

The Wetzel formulation (2) was applied to the data obtained for each child in each group during the twenty-month supplementation period and a twelve-month post supplementation period. The most prevalent change noted in the Grid patterns of the children receiving the added milk was an increase in the rate of development, followed in frequency by an increase in the speed of development, an improvement in the direction of development, and an improvement in the quality of growth. In almost all instances the children receiving the supplements underwent regressions in their Grid curves after the supplements were discontinued.

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In the reported Wetzel Grid findings, height and weight were treated as a single function, i.e., developmental level. To aid in the assessment of the effects of the milk supplements on the growth progress of the children with nutritive failure, it seemed desirable to study the degree to which height per se and weight per se were affected by the addition of known amounts of essential nutrients to the daily dietaries (Table I). In this study, therefore, the height and

TABLE I
NUTRIENTS SUPPLIED BY THE DRY MILK SUPPLEMENTS

Nutrient	Dry Nonfat milk (3 ounces)	Dry whole milk (4¼ ounces)
Calories	305.6	600.3
Protein	31.4 grams	31.8 grams
Calcium	1.1 grams	1.16 grams
Phosphorus	0.64 grams	0.92 grams
Iron		
Vitamin A		1,313.9 I. U.
Thiamine	.3 mg.	.32 mg.
Riboflavin	1.73 mg.	1.78 mg.
Niacin	.79 mg.	.82 mg.
Vitamin C		11.7 mg.

weight changes which occurred in the 82 children during twenty-month periods before, during and after the administration of the supplements have been computed, compared and evaluated.

METHOD OF STUDY

In the Nutrition Clinic, height is measured with a Baldwin Paper Measuring Scale (3). Shoes are removed, and the readings are taken in the standing position with the feet together and the heels, buttocks, shoulders and head touching the scale. Weight is determined with a Continental Scale after the child has removed his shoes and outer clothing.

In almost all instances the 82 children were weighed and measured at three-month intervals during the twenty-month period preceding the administration of the supplements and at two-month intervals during the milk and post milk periods. In sixteen of the test cases (children receiving the supplements) and seventeen of the control cases (children not receiving the supplements), height and weight measurements were not available for the entire twenty-month pre milk period. In each such instance, the records for each pair of children were evaluated in terms of the lesser period; thus the

time for which data were available for both the test and control child in each pair was identical. Similar adjustments were made for four test cases and eight control cases in the post milk period. One child in the control group did not return for examination during the entire post milk period so that only forty children comprised the control group during this phase of the study.

The height and weight values obtained for each child at each examination were compared with those listed in the height-weight tables of Simmons (4). To permit comparable treatment of the data, the differences between the attained values and the standard mean values were computed and then divided by the standard deviations listed in the Simmons tables for each age and for each sex. The results were plotted on individual Fels Composite Sheets (5), each child thereby serving as his own standard of comparison. The difference between the plotted points and the standard line of reference comprised the height and weight scores, the unit of measurement being the standard deviation. The frequency and magnitude of change in the height and weight scores at the end of each twenty-month period were compiled, and comparisons were made of the progress shown by each child as well as by each group of children during each twenty-month period. The frequency of gains in height or weight in amounts sufficient to decrease the deviations from the standard was subjected to statistical analysis, and significance determined in terms of sigma and chi square (6).*

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*Sigma (σ): The probability of occurrence of statistical deviations of different magnitudes relative to the standard deviation.

σ = standard error = $\sqrt{pq/n}$
σ_t = standard error for test cases
σ_c = standard error for control cases
p = percentage of universe
q = 100 — p
n = size of sample

$\sigma_{diff.} = \sqrt{\sigma_t^2 - \sigma_c^2}$ = standard error of difference between observed percentages for test and control cases.

$$\frac{\text{observed difference in per cent}}{\text{standard error of difference in per cent}} = \text{Sigma}$$

Chi Square (x^2): The ratio of an observed sum of squares to the corresponding variance fixed by hypothesis.

Observations

For ease of presentation the findings derived from an analysis of the height and weight data of each child during each period of study have been grouped as follows: (1) Distribution of Height and Weight Scores; (2) Frequency of Change in Direction of Height and Weight Scores; (3) Statistically Significant Differences in the Frequency of Increase in Height and Weight Scores; (4) Magnitudes of Change in Height and Weight Scores; and (5) Mean Height and Weight Increments.

Distribution of Height and Weight Scores

Figures I A, B, C, and D contain a summation of the height and weight scores of the children in the test and control groups at the beginning of the study and at the end of each twenty-month interval. It will be noted that 317 of the 327 height scores, and 325 of the 327 weight scores fell between zero (corresponding to the standard line of reference) and minus three and one-half standard deviations. In general, weight scores were somewhat closer to the standard values than were height scores, and the height and weight scores of the boys exceeded those of the girls at each of the four readings.

Frequency of Change in Direction of Height and Weight Scores

Increase in Either Height Score or Weight Score: During the pre milk period 21 (51.2 per cent) of the children in the test group and 25 (61.0 per cent) of the children in the control group gained height, and 22 (53.7 per cent) of the children in the test group and 26 (63.4 per cent) of the children in the control group gained weight in amounts sufficient to improve their height and weight scores. Gains in height of a magnitude great enough to produce a similar

Example:

		1st Attribute		
		+	—	Totals
2nd Attribute	+	a	b	(a + b)
	—	c	d	(c + d)
	Totals	(a + c)	(b + d)	(a + b + c + d) = N

$$x^2 = \frac{(ad - bc)^2 N}{(a + b)(c + d)(a + c)(b + d)}$$

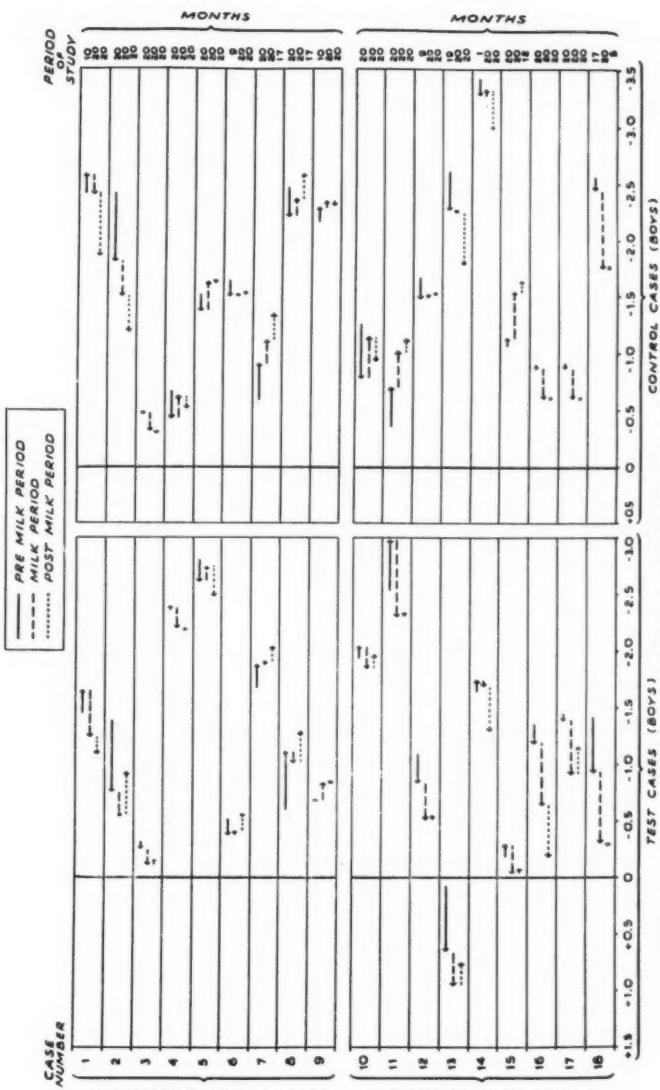

FIGURE I-A

The changes in the height scores of 18 test boys and 18 control boys during each period of study.

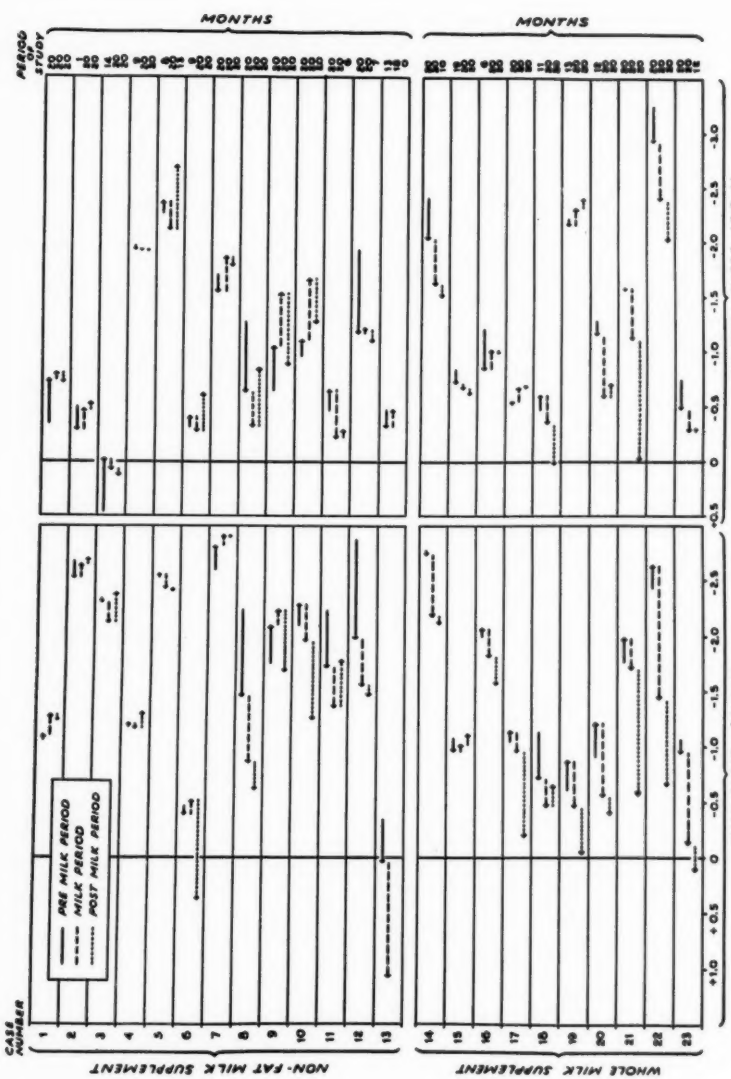

FIGURE I-B

The changes in the height scores of 23 test girls and 23 control girls during each period of study. One girl in the control group did not return during the entire post milk period.

One girl in the control group did not return during the entire post milk period.

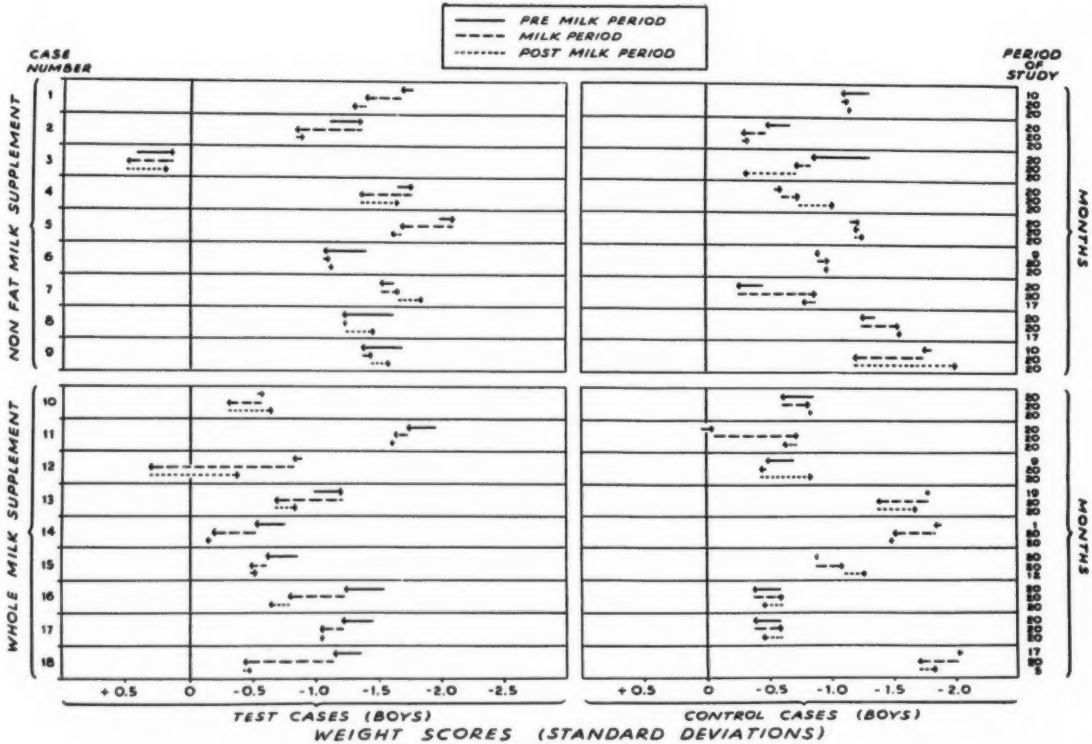

FIGURE I-C

The changes in the weight scores of 18 test boys and 18 control boys during each period of study.

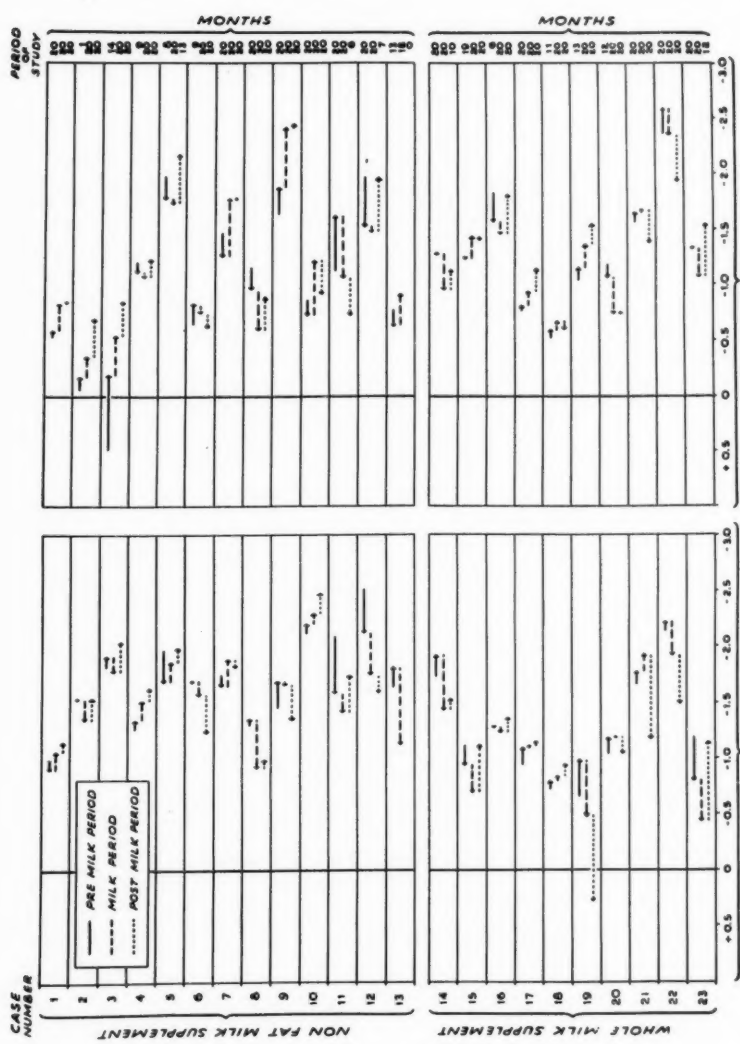

FIGURE I-D

The changes in the weight scores of 23 test girls and 23 control girls during each period of study. One girl in the control group did not return during the entire post milk period.

effect during the milk period were noted for 32 (78.1 per cent) of the test cases and 20 (48.8 per cent) of the control cases. Weight increases sufficient to increase the weight scores during this period occurred in 28 (68.3 per cent) of the test group and 18 (43.9 per cent) of the control group. During the post milk period 23 (56.1 per cent) of the test cases and 20 (50.0 per cent) of the control cases increased their height scores, and 14 (34.2 per cent) of the test cases and 14 (35.0 per cent) of the control cases increased their weight scores (Fig. I A, B, C, D).

Increase in Both Height and Weight Scores: Eleven (26.8 per cent) of the children in the test group increased in both their height and weight scores during the pre milk period. Twenty-four (58.5 per cent) underwent similar changes during the milk period, and 13 (31.7 per cent) during the post milk period. The corresponding numbers for the control group were 18 (43.9 per cent) in the pre milk period, 13 (31.7 per cent) in the milk period, and 7 (17.5 per cent) in the post milk period.

Increase in Height Score and Decrease in Weight Score: Ten (24.4 per cent) of the children in the test group improved their height scores but deviated further from the weight standards during the pre milk period, 8 (19.5 per cent) during the milk period, and 10 (24.4 per cent) during the post milk period. In the control group similar changes were made by 6 (14.9 per cent) of the children during the pre milk period, 5 (12.2 per cent) during the milk period, and 12 (30.0 per cent) during the post milk period.

Increase in Weight Score and Decrease in Height Score: Eleven (26.8 per cent) of the children in the test group improved their weight scores but deviated further from the height standards during the pre milk period, 4 (9.8 per cent) during the milk period, and 1 (2.4 per cent) during the post milk period. The numbers of children in the control group undergoing similar changes were 8 (19.5 per cent) in the pre milk period, 7 (17.1 per cent) in the milk period, and 5 (12.5 per cent) in the post milk period.

Decrease in Both Height and Weight Scores: Nine (22.0 per cent) of the children in the test group deviated further from the height and weight standards during the pre milk period, 5 (12.2 per cent) during the milk period, and 17 (41.2 per cent) during the post milk period. The corresponding numbers for the children in the control group were 8 (19.5 per cent) in the pre milk period, 16 (40.0

One girl in the control group did not return during the entire post milk period.

per cent) in the milk period, and 11 (27.5 per cent) in the post milk period.

Statistically Significant Differences in the Frequency of Increase in Height and Weight Scores

The results of the statistical analysis of the frequency of gains in height and weight in each group of children in amounts sufficient to increase the height and weight scores during each phase of the study are shown in Table II A. Comparisons were made between the test and control groups for each period, and between the pre milk and milk periods, milk and post milk periods, and pre milk and post milk periods for each group of children. Comparisons were made also on the basis of sex and type of milk contained in the supplement. Sigma values of 2 or over and chi square values of 4 or over were regarded as indicative of statistical significance.

Statistically significant differences were observed between the following:

Pre Milk Period:

(1) The number of boys in the test group who improved in weight score as compared with the number of girls in this group showing a similar change.

(2) The number of boys in the control group who improved in weight score as compared with the number of girls in this group showing a similar change.

(3) The number of boys in the test and control groups combined who improved in weight score as compared with the number of girls in both groups showing a similar change.

Milk Period:

(1) The number of test cases who improved in both height and weight scores as compared with the number of control cases showing similar changes.

(2) The number of boys in the test group who improved in both height and weight scores as compared with the number of boys in the control group showing similar changes.

(3) The number of children in the test group who received whole milk supplements and who improved in height score as compared with the number of children who received nonfat milk supplements and showed a similar change.

TABLE II A

STATISTICAL EVALUATION OF THE FREQUENCY OF GAINS IN HEIGHT AND WEIGHT IN AMOUNTS SUFFICIENT TO DECREASE DEVIATION FROM STANDARD BY THE VARIOUS GROUPS DURING EACH PERIOD OF STUDY*

	Chi Square Values in Various Periods of Study						Sigma Values in Various Periods of Study					
	Pre Milk		Milk		Post Milk		Pre Milk		Milk		Post Milk	
Comparative Groups	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
Test Children vs. Control Children	.29 (C)	.80 (C)	7.57 (T)	4.95 (T)	.30 (T)	.007 (C)	.89 (C)	.90 (C)	2.88 (T)	2.29 (T)	.55 (T)	.08 (C)
Test Boys vs. Control Boys	.47 (C)	.64 (C)	5.44 (T)	5.44 (T)	.47 (C)	.50 (C)	.69 (C)	.81 (C)	2.57 (T)	2.57 (T)	.69 (C)	.71 (C)
Test Girls vs. Control Girls	.35 (C)	.35 (C)	2.47 (T)	.79 (T)	1.84 (T)	.26 (T)	.59 (C)	.60 (C)	1.62 (T)	.90 (T)	1.55 (T)	.62 (T)
Test Boys vs. Test Girls	.24 (B)	4.45 (B)	.001 (G)	1.33 (B)	6.75 (G)	.58 (G)	.49 (B)	2.26 (B)	.04 (G)	1.20 (B)	2.82 (G)	.77 (G)
Control Boys vs. Control Girls	.44 (B)	5.49 (B)	1.26 (G)	.33 (G)	.40 (G)	.22 (B)	.67 (B)	2.60 (B)	1.14 (G)	.58 (G)	.49 (G)	.56 (B)
Boys vs. Girls	.66 (B)	9.79 (B)	.71 (G)	.13 (B)	5.25 (G)	.04 (G)	.82 (B)	3.41 (B)	.85 (G)	.36 (B)	2.22 (G)	.21 (G)
Whole Milk vs. Nonfat Milk	.63 (W)	.26 (W)	5.76 (W)	1.86 (W)	.05 (W)	.11 (W)	.80 (W)	.51 (W)	2.72 (W)	1.41 (W)	.22 (W)	.34 (W)

*Symbols contained in parentheses denote the group in which the frequency of gain exceeded that of the group to which it was compared: (T) = Test; (C) = Control; (B) = Boys; (G) = Girls; (W) = Whole Milk.

Post Milk Period:

(1) The number of girls in the test group who improved in height score as compared with the number of boys in this group showing a similar change.

(2) The number of girls in both groups combined who improved in height score as compared with the number of boys in both groups showing a similar change.

Statistically significant differences were observed also for (a) the number of children in the test group who showed a reduction in deviation in height during the milk period as compared with the number showing a similar change during the pre milk period; (b) the number of children in the test group who showed a reduction in deviation in both height and weight during the milk period as compared with that of the post milk period; and (c) the number of children in the control group who showed a reduction in deviation in weight during the pre milk period as compared with that of the post milk period (Table II B).

Magnitude of Change in Height and Weight Scores

The magnitude of the changes in the height and weight scores for each child during each period of study are shown in Figures I A, B, C, D. The magnitudes are recorded in terms of the standard deviation or parts thereof. The distribution of the individual changes in height and weight scores during each phase of the study, arranged in the order of increasing magnitude, is presented in Figures II A, B, C.

Pre Milk Period: 51.2 per cent (10 boys and 11 girls) of the children in the test group, and 60.9 per cent (12 boys and 13 girls) of the children in the control group increased in height in amounts sufficient to produce a mean gain in height score of 0.30 and 0.25 standard deviations respectively. Corresponding values for the weight scores in 53.6 per cent (13 boys and 9 girls) of the test group, and in 63.4 per cent (15 boys and 11 girls) of the control group were 0.22 and 0.16 standard deviations respectively. In the same period, 48.8 per cent (8 boys and 12 girls) of the children in the test group, and 39.0 per cent (6 boys and 10 girls) of the children in the control group did not maintain their rate of height gain, with the result that they underwent a mean loss in height score of 0.18 and 0.19 standard deviations respectively. Corresponding values for the weight scores in 46.3 per cent (5 boys and 14 girls) of the test group, and in 34.1

TABLE II B

STATISTICAL EVALUATION OF THE FREQUENCY OF GAINS IN HEIGHT AND WEIGHT OF TEST AND CONTROL CHILDREN IN AMOUNTS SUFFICIENT TO DECREASE DEVIATION FROM STANDARD IN EACH PERIOD OF STUDY*

	Chi Square Value								Sigma Value							
	Test				Control				Test				Control			
Comparative Periods:	Ht.		Wt.		Ht.		Wt.		Ht.		Wt.		Ht.		Wt.	
Pre Milk Period vs. Milk Period	6.46	(M)	1.85	(M)	1.23	(Pr.)	3.14	(Pr.)	2.64	(M)	1.32	(M)	1.12	(Pr.)	1.80	(Pr.)
Milk Period vs. Post Milk Period	4.47	(M)	9.53	(M)	.01	(Po.)	.67	(M)	2.17	(M)	3.16	(M)	.11	(Po.)	.82	(M)
Pre Milk Period vs. Post Milk Period	.20	(Po.)	3.17	(Pr.)	.10	(Pr.)	6.54	(Pr.)	.44	(Po.)	1.82	(Pr.)	1.00	(Pr.)	2.67	(Pr.)

*Symbols contained in parentheses denote the period in which the frequency of gain exceeded that of the period to which it was compared:

(Pr.) = Pre Milk; (M) = Milk; (Po.) = Post Milk.

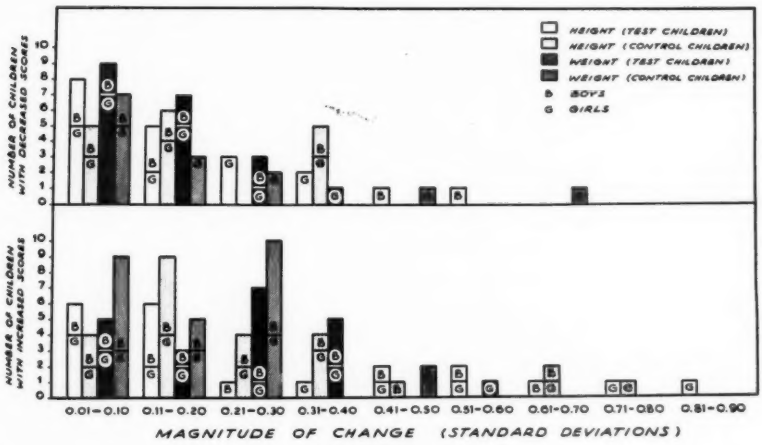

FIGURE II-A

The distribution of the changes in height and weight scores of 41 test children and 41 control children during the pre milk period. The scores are arranged in order of increasing magnitude.

per cent (2 boys and 12 girls) of the control group were 0.15 and 0.17 standard deviations respectively.

Milk Period: 78.0 per cent (14 boys and 18 girls) of the children in the test group, and 48.8 per cent (7 boys and 13 girls) of the children in the control group improved their height scores, the mean gain being 0.39 and 0.28 standard deviations respectively. Increases in weight score occurred in 68.3 per cent (14 boys and 14 girls) of the test cases, and in 43.9 per cent (7 boys and 11 girls) of the control group, the mean gain being 0.36 and 0.22 standard deviations respectively. During the same period, 21.9 per cent (4 boys and 5 girls) of the test group, and 51.2 per cent (11 boys and 10 girls) of the control group underwent a mean decrease of 0.09 and 0.20 standard deviations respectively in their height scores. Decreased weight scores occurred in 31.7 per cent (4 boys and 9 girls) of the test group, and in 56.1 per cent (11 boys and 12 girls) of the control group, the mean loss being 0.10 and 0.28 standard deviations respectively.

Of the 32 children in the test group who improved their height scores, 14 (5 boys and 9 girls) received nonfat milk, and 18 (9 boys and 9 girls) received whole milk. Of the 28 children in the test group who improved their weight scores, 13 (5 boys and 8 girls) received

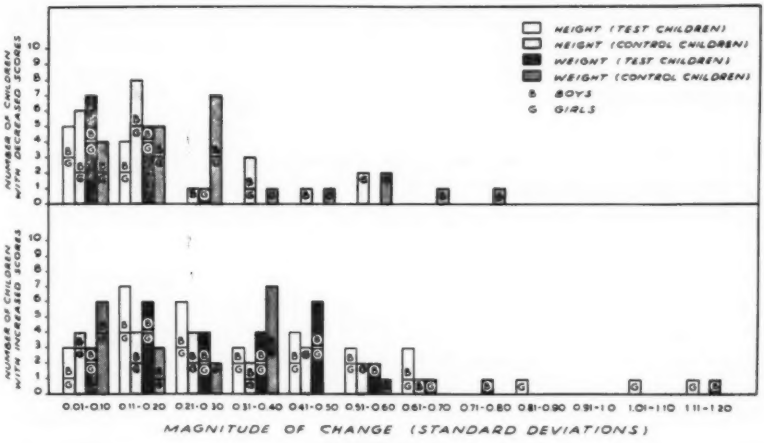
HEIGHT (TEST CHILDREN)
HEIGHT (CONTROL CHILDREN)
WEIGHT (TEST CHILDREN)
WEIGHT (CONTROL CHILDREN)
B BOYS
G GIRLS
NUMBER OF CHILDREN WITH DECREASED SCORES
NUMBER OF CHILDREN WITH INCREASED SCORES
0.01-0.10 0.11-0.20 0.21-0.30 0.31-0.40 0.41-0.50 0.51-0.60 0.61-0.70 0.71-0.80 0.81-0.90 0.91-1.0 1.01-1.10 1.11-1.20
MAGNITUDE OF CHANGE (STANDARD DEVIATIONS)

FIGURE II-B

The distribution of the changes in height and weight scores of 41 test children and 41 control children during the milk period. The scores are arranged in order of increasing magnitude.

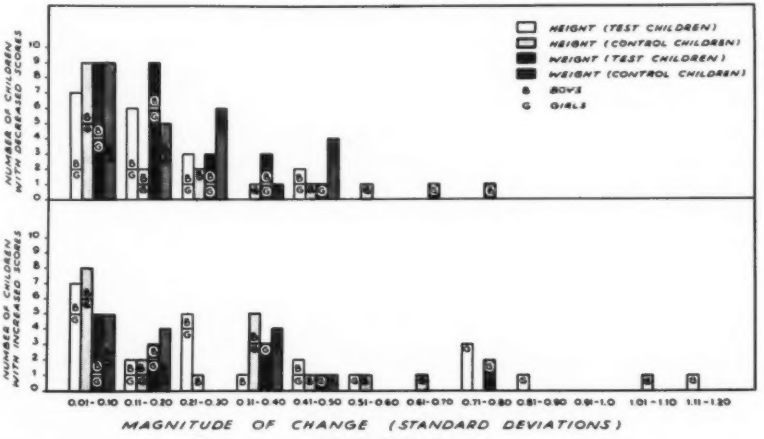
HEIGHT (TEST CHILDREN)
HEIGHT (CONTROL CHILDREN)
WEIGHT (TEST CHILDREN)
WEIGHT (CONTROL CHILDREN)
B BOYS
G GIRLS
NUMBER OF CHILDREN WITH DECREASED SCORES
NUMBER OF CHILDREN WITH INCREASED SCORES
0.01-0.10 0.11-0.20 0.21-0.30 0.31-0.40 0.41-0.50 0.51-0.60 0.61-0.70 0.71-0.80 0.81-0.90 0.91-1.0 1.01-1.10 1.11-1.20
MAGNITUDE OF CHANGE (STANDARD DEVIATIONS)

FIGURE II-C

The distribution of the changes in height and weight scores of 41 test children and 40 control children during the post milk period. The scores are arranged in order of increasing magnitude. In 4 control cases no change in height score was noted, and in 1 control case no change in weight score was noted.

nonfat milk, and 15 (9 boys and 6 girls) received whole milk. Of the 9 who lost in height score, 8 (4 boys and 4 girls) received nonfat milk, and 1 girl received whole milk. Of the 13 who decreased their weight score, 9 (4 boys and 5 girls) received nonfat milk, and 4 girls received whole milk.

Post Milk Period: 56.1 per cent (6 boys and 17 girls) of the children in the test group, and 47.5 per cent (8 boys and 11 girls) of the children in the control group underwent a mean gain in their height scores of 0.34 and 0.27 standard deviations respectively. A gain in weight scores occurred in 34.1 per cent (5 boys and 9 girls) of the test group, and in 35.0 per cent (7 boys and 7 girls) of the control group the mean gain being 0.27 and 0.19 standard deviations respectively. A mean loss of 0.16 standard deviations was noted for 43.9 per cent (12 boys and 6 girls) of the children in the test group, and for 42.5 per cent (7 boys and 10 girls) of the children in the control group. Corresponding values in weight scores in 65.9 per cent (13 boys and 14 girls) of the test group, and in 62.5 per cent (11 boys and 14 girls) of the control group were 0.20 and 0.21 standard deviations respectively.

Of 23 children in the test group who improved in height score, 12 (3 boys and 9 girls) received nonfat milk, and 11 (3 boys and 8 girls) received whole milk during the supplementation period. Of the 14 who gained in height score, 7 (2 boys and 5 girls) received nonfat milk, and 7 (3 boys and 4 girls) received whole milk during the milk period. Of the 18 children in the test group who showed a decrease in height score, 10 (6 boys and 4 girls) had received nonfat milk, and 8 (6 boys and 2 girls) had received whole milk. Of the 27 children in the test group who showed a decrease in weight score, 15 (7 boys and 8 girls) had received nonfat milk, and 12 (6 boys and 6 girls) had received whole milk.

Mean Height and Weight Increments

Pre Milk Period: The 41 children in the test group showed a mean height increment of 8.03 cm. during the pre milk period as compared with a mean gain of 8.41 cm. for the 41 children in the control group. The mean weight gains during this period were 3.82 kgm. for the children in the test group, and 4.00 kgm. for those in the control group. The mean gains in height and weight of the children in the control group during this period exceeded those of the children in the test group

by 0.38 cm. and 0.18 kgm. respectively (Table III). On the basis of height gained per month, the children in the test group averaged 0.50 cm., and those in the control group averaged 0.53 cm. The weight gain per month averaged 0.24 kgm. for the children in the test group, and 0.25 kgm. for those in the control group.

Milk Period: During the milk period, the children in the test group showed a mean height gain of 10.38 cm., and a mean weight gain of 6.12 kgm. In contrast, the children in the control group showed a mean height gain of 9.15 cm., and a mean weight gain of 4.77 kgm. The mean height and weight gains of the children in the test group exceeded those of the children in the control group by 1.23 cm. and 1.35 kgm. respectively. The children who received the supplement of whole milk during this period showed mean height and weight gains of 11.28 cm. and 7.09 kgm. respectively, as compared with mean gains of 9.60 cm. and 5.28 kgm. for the children who received the nonfat milk supplement. The mean monthly gains in height and weight for the children in the test group were 0.52 cm. and 0.31 kgm. respectively. Corresponding values for the children in the control group were 0.46 cm. and 0.24 kgm.

Post Milk Period: In the post milk period, the mean height and weight increments for the children in the test group were 7.71 cm. and 4.46 kgm. respectively. The corresponding values for the children in the control group were 7.70 cm. and 4.30 kgm. The mean height gains of both groups of children were approximately equal during this period. The mean weight gain of the children in the test group exceeded that of the children in the control group by 0.16 kgm. The mean gains per month in height and weight of the children in the test group were 0.43 cm. and 0.25 kgm. respectively. Corresponding values for children in the control group were 0.43 cm. and 0.24 kgm. respectively.

In comparing the mean height and weight increments of each group of children during each period of study, it was observed that the mean monthly height gain of the children in the test group during the milk period exceeded that attained during the pre milk and post milk periods by 0.018 cm. and 0.088 cm. respectively. The mean monthly weight gain during the milk period exceeded that made during the pre milk and post milk periods by 0.067 kgm. and 0.057 kgm. respectively. The mean monthly height gain of the children in the control group during the pre milk period exceeded that of the milk and post

TABLE III

MEAN HEIGHT AND WEIGHT INCREMENTS OF 41 TEST CHILDREN AND 41 CONTROL CHILDREN DURING EACH PERIOD OF STUDY

Group	Height						Weight					
	Total Gain		Average/Child		Average Gain/Month		Total Gain		Average/Child		Average Gain/Month	
	Cm.	Inches	Cm.	Inches	Cm.	Inches	Kgm.	Pounds	Kgm.	Pounds	Kgm.	Pounds
Pre Milk Period												
Test:												
Boys	147.2	57.95	8.18	3.22	.504	.198	65.17	143.37	3.62	7.96	.223	.491
Girls	182.0	71.65	7.91	3.11	.499	.196	91.48	201.26	3.98	8.75	.250	.551
Control:												
Boys	154.1	60.67	8.56	3.37	.528	.208	74.72	164.38	4.15	9.13	.256	.563
Girls	190.9	75.16	8.30	3.27	.523	.206	89.33	196.53	3.88	8.54	.244	.538
Test:												
Boys + Girls	329.12	129.60	8.03	3.16	.501	.197	156.65	344.63	3.82	8.41	.239	.525
Control:												
Boys + Girls	345.0	135.83	8.41	3.31	.525	.207	164.05	360.90	4.00	8.80	.249	.549
Milk Period												
Test Boys:												
Whole Milk	99.7	39.25	11.77	4.63	.554	.218	70.40	154.88	7.82	17.21	.391	.860
Nonfat Milk	87.1	34.29	9.68	3.81	.484	.191	38.48	84.65	4.27	9.40	.214	.470
Test Girls:												
Whole Milk	114.6	45.12	14.60	5.75	.573	.226	64.32	141.51	6.43	14.15	.321	.707
Nonfat Milk	124.0	48.82	9.54	3.76	.478	.188	77.73	171.01	5.98	13.15	.299	.658
Test Boys + Test Girls:												
Whole Milk	214.3	84.37	11.28	4.44	.564	.222	134.72	296.39	7.09	15.60	.354	.780
Nonfat Milk	211.1	83.11	9.60	3.78	.480	.189	116.21	255.66	5.28	11.62	.264	.581
Control:												
Boys	164.7	64.84	9.15	3.60	.457	.180	79.50	174.91	4.42	9.72	.221	.486
Girls	210.6	82.91	9.16	3.61	.458	.180	116.26	255.77	5.05	11.12	.253	.556

TABLE III (*continued*)

Group	Height						Weight					
	Total Gain		Average/Child		Average Gain/Month		Total Gain		Average/Child		Average Gain/Month	
	Cm.	Inches	Cm.	Inches	Cm.	Inches	Kgm.	Pounds	Kgm.	Pounds	Kgm.	Pounds
Milk Period												
Test:												
Boys + Girls	425.4	167.48	10.38	4.09	.519	.204	250.93	552.05	6.12	13.46	.306	.673
Control:												
Boys + Girls	375.3	147.76	9.15	3.60	.458	.180	195.76	430.68	4.77	10.50	.239	.525
Post Milk Period												
Test Boys:												
Whole Milk	66.0	25.98	7.33	2.89	.420	.165	39.33	86.52	4.25	9.61	.250	.551
Nonfat Milk	70.5	27.76	7.83	3.08	.410	.161	35.68	78.49	3.96	8.72	.207	.456
Test Girls:												
Whole Milk	78.4	30.87	7.84	3.09	.321	.170	52.27	114.99	5.23	11.50	.286	.632
Nonfat Milk	93.4	36.77	7.78	3.06	.456	.179	51.35	112.98	4.28	9.41	.250	.551
Test Boys + Test Girls:												
Whole Milk	144.4	56.85	7.60	2.99	.426	.168	91.60	201.51	4.82	10.60	.269	.593
Nonfat Milk	163.9	64.53	7.80	3.07	.435	.171	87.03	191.47	3.95	8.70	.232	.514
Control:												
Boys	141.8	55.83	7.88	3.10	.431	.170	78.35	172.37	4.35	9.58	.238	.524
Girls	166.1	65.40	7.55	2.97	.429	.169	93.63	205.98	4.26	9.38	.242	.532
Test:												
Boys + Girls	308.3	121.38	7.71	3.03	.431	.170	178.63	392.98	4.46	9.82	.249	.549
Control:												
Boys + Girls	307.9	121.22	7.70	3.03	.430	.169	171.98	378.35	4.30	9.46	.240	.528

milk periods by 0.077 cm. and 0.095 cm. respectively. The mean monthly weight gain of this group during the pre milk period exceeded that of the other two periods to a very slight extent, the average being 0.01 kgm. over that of the milk period, and 0.009 kgm. over that of the post milk period.

Discussion

Height and weight tables derived from the measurements obtained from a group of children with comparable genetic and environmental backgrounds are wholly valid only for children similar in all respects to the group from which the standards were developed. The children included in this study differed from the Brush Foundation group which served as the basis for Simmons' tables in many respects. However, since the milk study group was comprised of children who were all natives of north-central Alabama and who had similar genetic and environmental backgrounds, it was felt that any errors inherent in the application of the height and weight readings from this homogeneous group of children to standards derived from another homogeneous group of children would be relatively constant.

As is evident from the distribution of height and weight scores (Fig. I A, B, C, D), almost all of the children selected for this study were lean and short for their age, an observation supported by clinical and Wetzel Grid findings (7). The demonstration that a significantly greater number of children receiving the milk supplements decreased their deviations from the height and weight standards during the milk period as contrasted with the number of children in the control group undergoing a similar change, and as compared with the progress made by the children in the test group during the periods immediately preceding and following the administration of the supplements, suggests that chronic undernutrition was one of the prime underlying causes of the poor growth progress of these children.

It is of interest that the children receiving the supplements gained an average of 1.23 cm. and 1.35 kgm. over and above the gains made by the children in the control group during the milk period. These amounts were equal to 11.8 per cent and 22.1 per cent respectively of the total mean height and weight gains made by the children in the test group during this phase of the study. The average monthly gain in height made by the children in the test group during the milk period exceeded the monthly gain made during the pre milk period by 3.6 per cent,

and that made during the post milk period by 20.4 per cent. The average monthly weight gain made by the children in the test group during the milk period exceeded that made during the pre milk period by 28.0 per cent, and that made during the post milk period by 22.9 per cent. During this period, the average monthly gains in height and weight made by the children in the test group who received the whole milk supplements exceeded those made by the children receiving the nonfat milk supplements by 17.5 per cent and 34.1 per cent respectively.

Except for calories, vitamin A and vitamin C, the nutrients supplied by both types of supplement were approximately the same (Table I). The whole milk supplement contained 296 calories, 1,314 I. U. vitamin A, and 11.7 mg. vitamin C more than did the nonfat milk supplement. Since the dietary findings (8) indicate that during the milk period, 72 per cent of the children receiving the milk supplements consumed less than 75 per cent of the recommended allowance for calories, 45 per cent consumed less than 75 per cent of the recommended allowance for vitamin A, and 85 per cent consumed less than 75 per cent of the recommended allowance for vitamin C when the nutritive value of the milk supplement was excluded from the dietary assessments, it would appear that the difference in the nutritive value of the two milk supplements may account, to some extent, for the greater height and weight gains made by the children receiving the whole milk as compared with those made by the children receiving the nonfat milk.

Previous studies have shown that episodes of retarded growth, as measured by the Wetzel Grid, are most common in children with chronic nutritive failure in north-central Alabama in June, July and August (9). The pre milk phase of this study extended from the last of July 1943 through March 1945, the milk period from April 1945 through November 1946, and the post milk period from December 1946 through July 1948. The measurements obtained at the beginning of the pre milk period were made at a time when many of the children were undergoing episodes of retarded growth. Since this phase included only one other slow-growing period, whereas the milk phase included two slow growing periods and the post milk phase included one and one-half slow growing periods, the height and weight gains made by the children in the test group during the period of supplementation are of added significance.

All reported observations (10) indicate that in the northern and southern hemispheres increases in weight are greatest in the fall and least in the spring, and increases in height are greatest in the spring and least in the fall. The factor of seasonal variation, as well as the increased nutrient intake, may account for the greater mean increase in weight as compared with the mean increase in height made by the children in the test group during the supplementation phase of the study.

Summary and Conclusions

1. Eighty-two children were selected on the basis of history or evidence suggestive of nutritional deficiency disease, freedom from allergy to cow's milk, freedom from contagious or infectious disease, and accessibility of their home. They were paired on the basis of sex, comparative roentgenograms of the left hand, wrist and forearm, general body type, and apparent prevailing phase of growth. One child in each pair received a dietary supplement of reliquefied milk solids, equivalent to one quart of whole milk or one quart of nonfat milk, six days a week for a period of twenty months. Twenty-two children (9 boys and 13 girls) received the nonfat milk, and 19 children (9 boys and 10 girls) received the whole milk.

2. Height and weight changes in the children receiving the supplements (test group) and in those not receiving the supplements (control group) were compared for twenty-month periods before, during, and after the administration of the milk.

3. During the milk period a significantly greater number of children in the test group improved in both their height and weight scores as compared with the number in the control group showing similar changes.

4. A significantly greater number of children in the test group improved their height scores during the milk period as compared with the number showing similar changes during the pre milk period and during the post milk period.

5. The children receiving the milk supplements gained an average of 1.23 cm. and 1.35 kgm. over and above the gains made by the children in the control group during the milk period. These amounts were equal to 11.8 per cent and 22.1 per cent of the respective mean height and weight gains made by the children in the test group during the supplementation phase of the study.

6. The average monthly gain in height made by the children in

the test group during the milk period exceeded that made during the pre milk period by 3.6 per cent, and that made during the post milk period by 20.4 per cent. In contrast, the average monthly height gain of the children in the control group during the milk period was 12.8 per cent less than that made during the pre milk period, and exceeded that made during the post milk period by 6.5 per cent.

7. The average monthly gain in weight made by the children in the test group during the milk period exceeded that made during the pre milk period by 28.0 per cent, and that made during the post milk period by 22.9 per cent. In contrast, the average monthly gain in weight made by the children in the control group during the milk period was 4.0 per cent less than that made during the pre milk period, and 0.4 per cent less than that made during the post milk period.

8. The average monthly gains in height and weight made by the children receiving the whole milk supplement during the milk period exceeded those made by the children receiving the nonfat milk supplement by 17.5 per cent and 34.1 per cent respectively. It is postulated that the greater calorie, vitamin A and vitamin C content of the whole milk supplement may have been responsible for the difference in these findings.

9. Although the height and weight of a growing child are interrelated, changes in either dimension may be of aid to the clinician in interpreting the effects of dietary supplements.

REFERENCES

1. SPIES, T D., & DREIZEN, S. 1949. The Effect of Milk Supplements on the Growth of Children with Nutritive Failure. *J. Pediat.*, **34**, 393.
2. WETZEL, N. C. 1948. The Treatment of Growth Failure in Children. NEA Service, Inc., Cleveland.
3. BALDWIN, B. T. 1921. Physical Growth of Children from Birth to Maturity. Univ. of Iowa Studies in Child Welfare **1**, No. 1.
4. SIMMONS, K. 1944. The Brush Foundation Study of Child Growth and Development. II. Physical Growth and Development. *Monog. Soc. for Research in Child Develop.* **9**, No. 1.
5. SONTAG, L. W., & REYNOLDS, E. L. 1945. The Fels Composite Sheet. I. A Practical Method for Analyzing Growth Progress. *J. Pediat.* **26**, 327.
6. PEARL, R. 1930. *Introduction to Medical Biometry and Statistics,* 2nd Ed. Rev. (Philadelphia: W. B. Saunders Co.).
7. MANN, A. W., DREIZEN, S., & SPIES, T. D. 1947. The Determination of Status and Progress in Children with Nutritive Failure. *J. Pediat.* **31**, 161.
8. Unpublished observations.
9. DREIZEN, S., MANN, A. W., & SPIES, T. D. 1948. Seasonal Incidence of Retarded Growth in Children with Nutritive Failure. *Lancet* **1**, 175.
10. NELSON, W. E. 1945. *Textbook of Pediatrics,* 4th Ed. Rev. (Philadelphia: W. B. Saunders Co.), p. 33.

Growth, 1950, **14**, 213-230.

GROWTH AND ARTICULAR CHANGES IN SLOWLY AND RAPIDLY DEVELOPING MICE FED A HIGH-FAT DIET

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In mice of strain C57 black fed a high-fat diet throughout life, the skeletal time curve was accelerated: As compared with the corresponding processes in mice kept on a regular diet, growth and development of the long bones were hastened, articular changes set in prematurely, and the incidence of osteoarthritis was increased. Males reacted more readily and more intensely than females. This sex difference was tentatively explained, as follows: The male skeleton ages more slowly than the female; moreover, the susceptibility of the skeleton to stimuli decreases with advancing age, a condition observed previously regarding the skeletal effects of hormones (Silberberg and Silberberg 1943, 1949, 1950).

If this interpretation was correct, the high-fat diet should modify skeletal growth and ageing less conspicuously in individuals of rapidly developing strains of mice, such as dba, than in those of slowly developing strains, such as C57 black. In order to further analyze the relationship between the rate of physiologic development and ageing and the skeletal response to stimuli, the following experiments were carried out.

MATERIAL AND METHODS

One hundred and ninety-five male mice of the closely inbred strain dba, raised in our laboratory, were used. The animals were four to five weeks old and weighed on the average 12 grams at the beginning of observations. *Control Series:* One hundred and six mice were fed a stock diet of Purina Laboratory Chow as libitum with

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water available at all times. This ration contains 5% fat. *Test Series:* Eighty-nine mice received a high-fat diet consisting of 75% laboratory Chow ground to a meal with 25% lard (Swift's "Silverleaf" Brand) added. This diet, details of which have been given previously, contains 29% fat (Silberberg and Silberberg 1950b); it is adequate in all respects and well tolerated by the animals. The mice were weighed at regular intervals. During the period of growth, mice of the control and experimental series were killed in groups of 4 or 6 each, after ½, 1, 2, 3, or 5 months of observation. Later on, samples of animals were sacrificed in 2 or 3 months' intervals. A number of mice were allowed to live to the end of their natural life, while others were killed, when their condition warranted it. According to their age at death, the following three age groups of adult animals were available for examination: Age group I: 7-12 months: 30 control and 25 test mice; age group II: 13-18 months: 40 control and 33 test mice; age group III: 19 months and over: 10 control and 5 test mice.

The material secured at necropsy was handled in the usual manner. The kneejoints including the lower end of the femur and the upper part of the tibia were removed as a whole, fixed and decalcified in Bouin's solution, and embedded in paraffin. Semiserial sections were stained with hematoxylin and eosin.

Gross Observations

The animals appeared healthy, and showed no signs of nutritional deficiencies. During the period of growth, the mean weights of the test animals lagged slightly behind those of the controls. However, at the age of six months, the mice of the two groups showed about equal weights. During the second year of life, the test animals weighed approximately 8% more than the controls. The individual weights of the test animals varied within a wider range than those of the controls. The following table gives the mean weights of the mice in the control and test series and the maximum and minimum deviations from these means.

Microscopic Examination

Growth Zones

Mice 1½ months old: In the controls, the width of the growth zones was about 165 microns, the single cartilage cell row being composed

TABLE 1
SHOWING THE MEAN WEIGHTS AND THE DEVIATIONS FROM THE MEANS IN CONTROL AND TEST ANIMALS

	CONTROLS		TESTS	
initial	12.2	max. d. 0.8; min. d. 2.2	12.4	max. d. 0.6; min. d. 2.4
2 months	15.2	max. d. 0.8; min. d. 1.2	15.3	max. d. 2.7; min. d. 0.3
3 months	17.2	max. d. 0.8; min. d. 1.2	16.2	max. d. 1.8; min. d. 1.2
4 months	19.2	max. d. 1.8; min .d. 1.2	18.6	max. d. 2.4; min. d. 1.6
5 months	19.2	max. d. 2.8; min. d. 0.2	17.9	max. d. 3.1; min. d. 0.9
6 months	19.3	max. d. 2.7; min. d. 0.3	19.6	max. d. 3.4; min. d. 1.6
7 months	19.2	max. d. 5.8; min. d. 3.2	20.5	max. d. 6.5; min. d. 5.5
8 months	19.2	max. d. 6.8; min. d. 2.2	21.3	max. d. 5.7; min. d. 3.3
9 months	19.1	max. d. 5.9; min. d. 4.1	21.0	max. d. 6.0; min. d. 5.0
10 months	21.0	max. d. 4.0; min. d. 3.0	20.9	max. d. 6.1; min. d. 4.9
11 months	21.3	max. d. 4.7; min. d. 2.3	24.0	max. d. 3.0; min. d. 8.0
12 months	21.8	max. d. 4.2; min. d. 2.8	22.3	max. d. 3.7; min. d. 5.3
13 months	22.3	max. d. 3.7; min. d. 3.3	21.6	max. d. 3.4; min. d. 4.6
14 months	22.1	max. d. 3.9; min. d. 2.1	22.5	max. d. 2.5; min. d. 5.5
15 months	22.1	max. d. 4.9; min. d. 1.1	24.0	max. d. 1.0; min. d. 6.0
16 months	23.3	max. d. 2.7; min. d. 7.3	24.0	max. d. 2.0; min. d. 5.0
17 months	23.8	max. d. 3.2; min. d. 0.8	22.0	max. d. 1.0; min. d. 1.0
18 months	22.6	max. d. 1.4; min. d. 2.6	24.0	max. d. 4.0; min. d. 3.0
19 months	23.2	max. d. 3.8; min. d. 3.2	24.0	max. d. 3.0; min. d. 2.0
20 months	22.0	max. d. 1.0; min. d. 1.0	22.0	max. d. 3.0; min. d. 5.0
21 months		—	24.0	max. d. 1.0; min. d. 1.0

of 7-9 columnar and 3-4 hypertrophic cells. In the test animals, the growth zones were about 150 microns wide, and the individual cartilage cell row contained 6-8 columnar and 2 or 3 hypertrophic cells. The cartilage cell rows showed regular arrangement; calcification of cartilage and the amount of chondromucoid groundsubstance were about the same in both groups of animals. In the test series, the primary spongiosa was coarser than in the controls.

Mice 2 months old: The growth zones of the controls measured about 150 microns, and in the single cartilage cell row 6-9 columnar and 2 or 3 hypertrophic cells were present (Fig. 1). In mice of the test series, the growth zones were narrowed down to about 130 microns, and in the single cartilage cell row 5-7 columnar and 1 or 2 small cells of hypertrophic type were counted. Hyalinization of the matrix and replacement of cartilage by bone were farther advanced in the test animals than in the controls (Fig. 2).

Mice 3 months old: In the control animals the growth zones were about 115 microns wide, and in the single cartilage cell row 5-6 columnar and 2 or 3 hypertrophic cells were found. The growth zones of the test animals measured only 75-90 microns, and the number of cells in the single cartilage cell row had decreased to 4-6 small col-

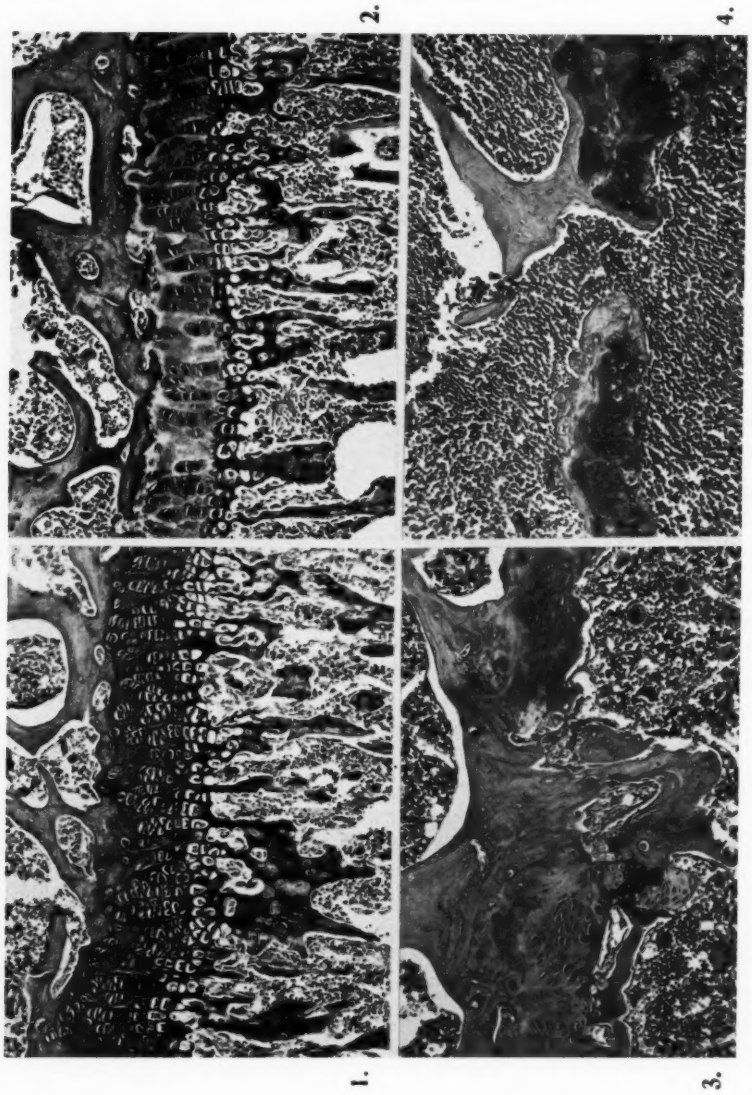
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PLATE I

Fig. 1-4. Sections through the growth zones at the upper end of the tibia of male mice of strain dba. x125.

Fig. 1. Two-month-old mouse fed the control diet. The cartilage cell rows are regularly arranged and separated from each other by thin layers of ground substance.

Fig. 2. Two-month-old mouse fed the high-fat diet since weaning. The growth zone is narrower, there are fewer hypertrophic cells and there is more ground substance between the cartilage cell rows, and the replacement of cartilage by bone takes place farther proximally than in the control.

Fig. 3. Six-month-old mouse fed the control diet. The epiphyseal cartilage is inactive and contains a thick osseous plug.

Fig. 4. Six-month-old mouse fed the high-fat diet. There is perforation of the inactive and partly ossified epiphyseal disk.

umnar and 1 or 2 small cells of hypertrophic type. In both groups of animals, regressive changes and destruction of cartilage had made progress, and ossified plugs of degenerated cartilage traversed the growth zone. Again these changes as well as metaphyseal and cortical bone formation were more accentuated in the test animals than in the controls.

Mice 4 months old: The growth zones of the controls were between 75 and 90 microns wide, and were composed of 5-7 small cells of columnar and hypertrophic type. The cartilage cell rows were separated from each other by large wedges of hyaline matrix and plugs of degenerated or ossified cartilage, that had increased in number and size as compared with the preceding stage. In animals of the test series, the growth zones were too irregular in width to be measured or to permit an exact cell count. Moreover, the distinction between columnar and hypertrophic cartilage became difficult. A transverse bony lamella indicative of cessation of growth delimited the epiphyseal cartilage from the metaphysis. The lamella as well as the cortical bone were thicker in animals of the test series than in the controls.

Mice 6 months old: In the controls, the growth zones consisted of sclerosed cartilage sealed off from the metaphysis by a bony plate. Thick osseous plugs showing beginning resorption by bone marrow were present (Fig. 3). In the test animals, the remnants of the epiphyseal cartilage appeared more inactive than in the controls, and epiphyseo-diaphyseal union had already occurred (Fig. 4). In both groups of animals, vigorous resorption had led to a thinning out or to complete resorption of the primary spongiosa.

At still later ages, the structure of the growth zones of the controls approached gradually that of the test animals, and ultimately hardly any differences were noticeable in the two groups of animals.

Joints: The findings, representative sections of which are shown (Figs. 5-10), were graded in accordance with criteria fully described previously (15b). Briefly, this is the classification:

No Change: There is the usual development from undifferentiated to hypertrophic cells and replacement of cartilage by bone. The ligaments, synovialis and capsule show regular architecture.

Age Changes: Slight changes consist of focal or diffuse hyperplasia and hypertrophy of the cartilage cells, while hematoxylin-eosin stains fail to disclose regressive changes in the matrix. In other animals,

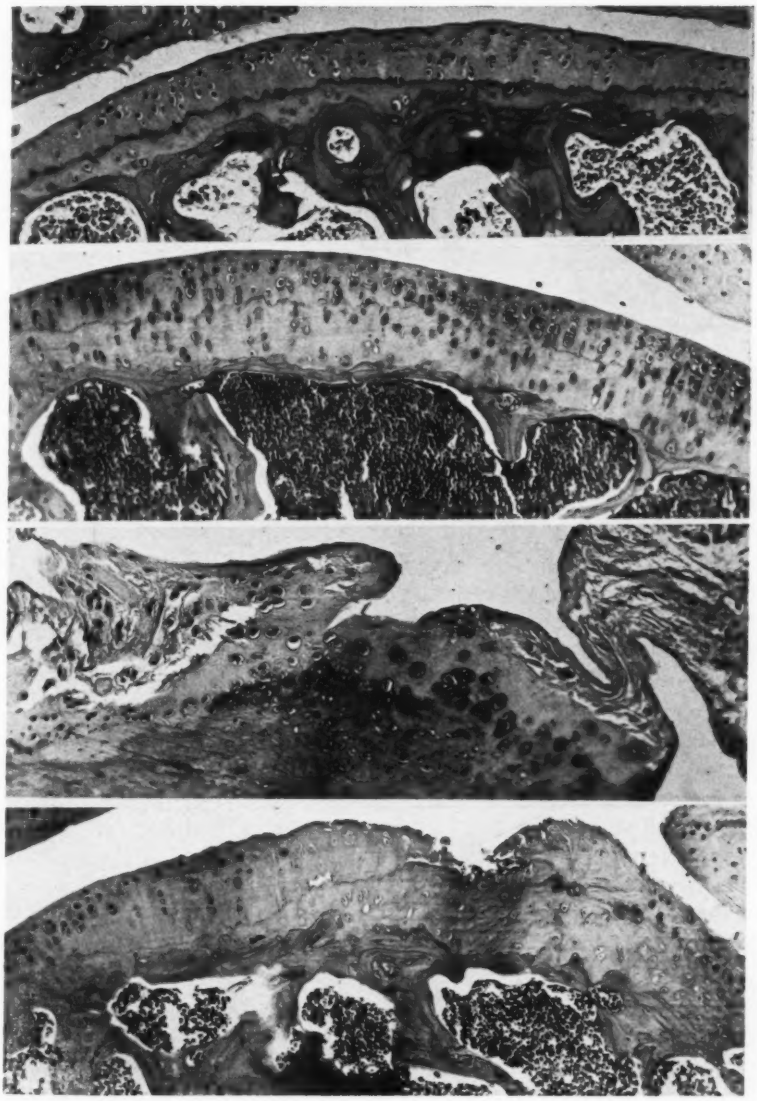


PLATE II

Fig. 5-8. Sections through the articular surface at the upper end of the tibia. x125.
Fig. 5. Resting articular surface.
Fig. 6. Articular surface showing proliferation of cartilage.
Fig. 7. Articular cartilage showing advanced growth.
Fig. 8. Ulcer at the surface with regressive changes in the cartilage at its edges.

the cartilage is resting, while the synovialis proliferates. The newly formed areolar tissue infiltrates into the epiphyseal bone marrow through enlarged vascular channels, and the ligaments undergo cartilagination.

Advanced age changes are characterized by marked proliferation or regressive changes in cartilage, ligaments or synovialis.

Osteoarthritis: There is a combination of growth and regressive changes associated with deformities of the joint: Focal or generalized growth of the cartilage, ulceration and eburnation of the articular surfaces, proliferation and fibrosis of the synovialis, fibrosis or cyst-formation of the bone marrow, cartilagination and degeneration of the lilgaments.

Results

Up to the age of six months, only slight and localized articular changes were found. In 3 of 12 controls, 4 and 6 months old, slight age changes were noted. In the test mice, joint changes were first seen at the age of three months: 3 of 18 animals, 3 to 6 months old showed slight, and 2 had developed advanced age changes.

Articular changes were seen more frequently from the second half of the first year on. The following table shows the grades and incidences of the articular findings in the adult animals.

At a mean age of 9.3 months more than one-half (56.0%) of the test animals had slight changes, but only about one-third (36.7%) of the controls—mean age 9.4 months—were thus affected. This increase had occurred at the expense of mice with resting joints, the latter being about three times as numerous (36.7%) in the controls as in the test series (12.0%). Towards the end of the first half of the second year of life, that is at the mean age of 16.1 and 17.6 months respectively, 51.5% of the test animals and 50.0% of the controls had developed slight age changes. Articular changes had thus remained stationary in the test animals but had made progress in the controls. In the animals 13 to 18 months old the high-fat diet had not significantly affected the course of degenerative joint disease. In the test mice, the incidence of advanced age changes was 9.1% and of osteoarthritis 33.3% as compared with a 12.5% incidence of advanced age changes and a 32.5 per cent incidence of osteoarthritis in the controls. In still older controls, the incidence of articular changes increased only slightly (20.0%) and osteoarthritic lesions

TABLE 2

SHOWING THE INCIDENCES OF THE ARTICULAR FINDINGS IN ANIMALS OF THE DIFFERENT AGE GROUPS

Age group months		Total No. of mice	Mean age at death months	No Change No. of mice	%	Sight Age Change No. of mice	%	Advanced Age Change No. of mice	%	Osteoarthritis No. of mice	%
7-12	Controls	30	9.4	11	36.7	11	36.7	5	16.6	3	10.0
	Tests	25	9.3	3	12.0	14	56.0	5	20.0	3	12.0
13-18	Controls	40	17.6	2	5.0	20	50.0	5	12.5	13	32.5
	Tests	33	16.1	2	6.1	17	51.5	3	9.1	11	33.3
19-21	Controls	10	19.7	1	10.0	5	50.0	2	20.0	2	20.0
	Tests	5	20.4	0	0.0	2	40.0	1	20.0	2	40.0
Irrespective of age	Controls	80	14.5	14	17.5	36	45.0	12	15.0	18	22.5
	Tests	63	14.0	5	7.9	33	52.4	9	14.3	16	25.4

even declined (20.0%). As had been observed in strain C57, there seems to be a peak in susceptibility to articular changes about late middle age. The 40% incidence of osteoarthritis in test mice over 19 months of age is inconclusive in view of the small number (5) of animals reaching this age.

A consideration of the various articular changes in controls and test mice irrespective of age discloses the following: Under the influence of the high-fat diet there occurred but a slight shift from unchanged joints to those with slight changes. The total incidence of osteoarthritis was the same in the control and test animals. The results thus support the view that the skeletal response to certain stimuli decreases with advancing physiologic age, and that the presence of age changes alone is not the only basis for the development of osteoarthritis. Apparently additional prerequisites must be fulfilled before the osteoarthritic changes take place.

Observations Concerning Strain Differences dba: C57 Black

The skeletal response to the high-fat diet of mice of strain dba is in striking contrast to results obtained in identical experiments carried out in mice of the slowly ageing strain C57 (Silberberg and Silberberg 1950b). In the latter, growth and development of the long bones were conspicuously accelerated, and the incidence of osteoarthritis increased twofold under the influence of the fat-enriched diet.

The strain differences are apparently not confined to the skeleton. As compared to controls fed a stock diet, mice of strain dba receiving the fat-enriched diet gained only slightly more weight—about 8%—than their controls, and showed hardly any increase in fat deposits, while in the corresponding animals of strain C57, there was as much as 100% gain in weight associated with the deposition of considerable amounts of body fat.

The two strains did not differ only in their reaction to the high-fat diet, but also in the microscopic appearances of the normal joints and in the development of spontaneous articular lesions. In strain dba, the bones were more delicate and the cartilage was less abundant than in strain C57. In the articular cartilage of adult and old mice of strain dba, growth was much less active and regressive changes were by far less extensive than in strain C57. In aged animals of the latter strain, ulceration of the articular surface with over-

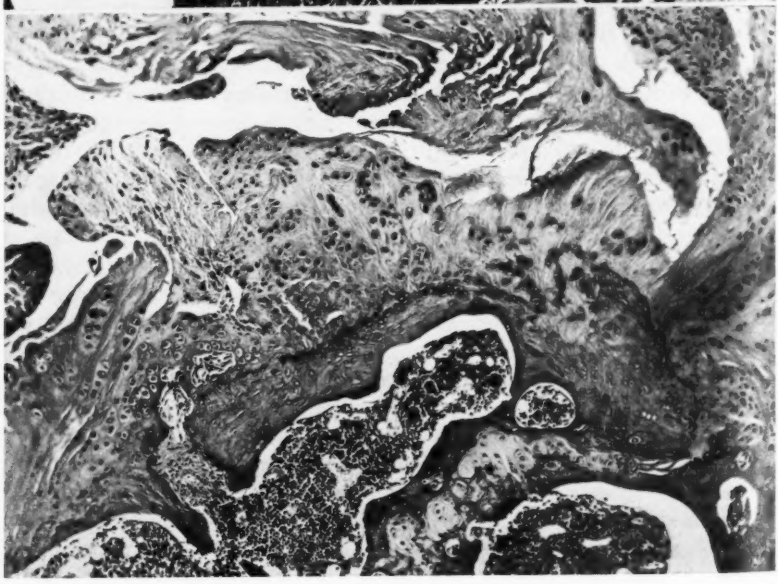
growth and eburnation were rather common, while this was a rare occurrence in strain dba. The synovialis on the other hand, reacted more conspicuously in the latter than in strain C57. In strain dba, joints showing primary synovial changes outnumbered those in which the cartilage was primarily altered. Conversely, in strain C57 joints with resting cartilage and proliferating synovialis were rare.

In addition, the thyroids of mice of strain dba showed signs of increased activity as compared with those of strain C57 (Figs. 11, 12). In the former, the acini were lined by high epithelium and contained softened scalloped colloid, while in the latter the acinar epithelium was cuboidal, and the colloid was dense without evidence of absorption. In adult mice of strain dba, the thyroids showed cysts filled with purplish secretions, an uncommon finding in strain C57 (Figs. 13, 14). Similar variations in the thyroid of mice of various strains have been noted by other investigators (Barry and Kennaway 1937, Loeb and Kirtz 1939, Grüneberg 1943, 1947).

Discussion

The present discussion has to revolve around two points: The strain differences in the spontaneous age changes, and those in the response to the high-fat diet. The first problem includes the question whether the relative resistance of the articular cartilage in mice of strain dba is a local phenomenon or whether it is controlled by extraarticular factors. The comparatively small amount of pre-existing cartilage in this strain may preclude an extensive growth response. The greater refractoriness of the cartilage may, however, also be due to differences in biochemical and biophysical composition. The latter will change in different strains depending on the rate of physiologic development and ageing which is in part genetically determined (Bauer and Bennett 1936, Loeb 1940, 1945, Silberberg and Silberberg 1943, 1949). Moreover, the composition of the cartilage, is probably also governed by extra-articular factors, one of them being the thyroid hormone. The appearance of the cartilage, the delicacy of the bony structures, and the scarcity of hyperostotic osteoarthritic lesions in mice of strain dba are consistent with increased activity of the thyroid. Thyroid hormone promotes resorption of bone; it also exerts a condensing effect on the matrix and might thus inhibit growth of cells as well as softening of the groundsubstance, both important steps in the pathogenesis of osteoarthritis. Estrogen, although inhibiting

9.



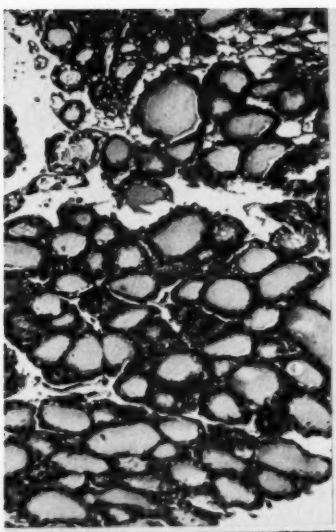
10

PLATE III

Fig. 5-10. Sections through the articular surface at the upper end of the tibia. x125.

Fig. 9. Osteoarthritic lesion at the surface of the upper end of the tibia with fibrosis of the bone marrow, proliferation of the cartilage, and cartilagination of the ligament. x150.

Fig. 10. Advanced osteoarthritis at the upper end of the tibia. x125.



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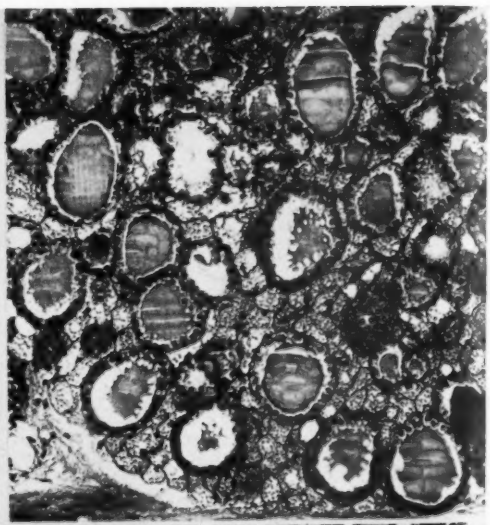

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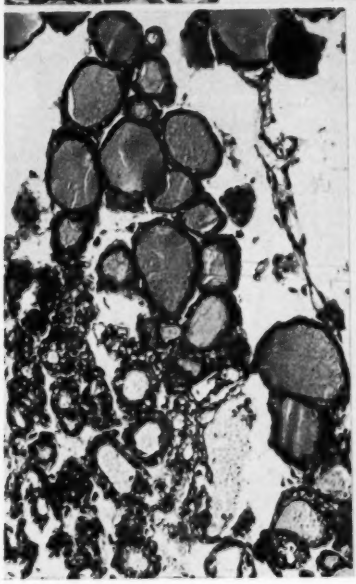

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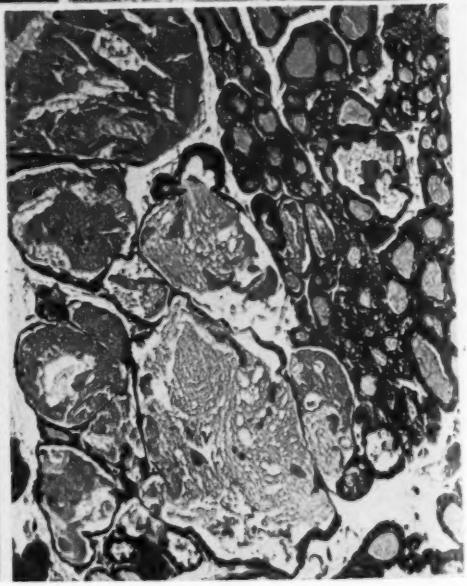

14.

PLATE IV

Fig. 11-14. Sections through the thyroid glands of normal male mice. x200.

Fig. 11 Mouse of strain C57 black, 2 months old. The acini are of usual size and contain hard colloid, the acinar epithelium is low cuboidal.

Fig. 12. Mouse of strain dba, 2 months old. The acini are large and lined by high epithelium, the colloid is soft and shows scalloping.

Fig. 13. Mouse of strain C57 black, 6 months old. The thyroid gland is resting and contains firm colloid, the acinar epithelium is low cuboidal.

Fig. 14. Mouse of strain dba, 6 months old. Large cysts with secretions are present.

resorptive processes, likewise causes condensation of the matrix (Silberberg and Silberberg 1943, 1949). However, this hormone is probably not involved in the present results, since males were used.

The observations of strain differences in the response to the high-fat diet may also help in the analysis of mechanisms involved in the spontaneous processes of ageing, the nature of which is still little understood. Thus, the differences in gain of body weight in the two strains point to differences in their fat metabolism. Possibly, mice of strain dba may be capable of metabolizing excessive fat, while mice of strain C57 are unable to do so. Reports concerning strain differences in various phases of metabolism appear in increasing numbers: Certain strains of rats vary regarding the lipotropic action of administered estrogen (György and Rose 1949). According to some investigators, mice with a high rate of mammary cancer—like strain dba—produce larger amounts of estrogen than do lower cancer strains such as strain C57 (Korteweg 1948). However, in adult females of strain dba, the slender build of the skeleton did not indicate hyperestrinization. In this strain thus—and possibly in contrast to the high mammary cancer strain C3H—the high breast cancer incidence may be due to increased tissue susceptibility rather than to increased production of estrogen. Moreover, various strains of mice differ in their requirements for vitamins (Fenton and Cowgill 1947), in their turnover of phosphorus (Johnson and Albert 1949) and iodine (Gross and Schwartz 1950), and in the lipid fractions contained in their adrenals (Vicari 1943, Vicari and Little 1945). Since thyroid hormone may act as a controlling agent in fat transfer (Handler 1948), increased thyroid activity in mice of strain dba may account for rapid breakdown of fat consumed in excess. Finally, vitamin A plays a rôle in both the metabolism of fat and in skeletal development (Eddy and Dalldorf 1944, Maddock, Wolbach and Maddock 1949) and also is closely related to thyroid activity (Heimer, Maslow and Sobel 1949). This vitamin may thus be likewise involved in both the spontaneous and the experimental strain differences, although its exact position in the chain of events is as yet unknown.

Summary

In mice of strain dba the long bones show a more delicate build, and the skeletal time curve is accelerated as compared with conditions

in strain C57 black. The two strains differ also in the structure of various endocrine glands. Differences in thyroid activity may be responsible for some of the skeletal strain characteristics and for variations in the skeletal time curve.

In mice of strain dba, skeletal growth and ageing were only slightly hastened, and the incidence of articular lesions was unaffected by feeding a fat-enriched diet throughout life. These results are considered to be related to local qualitative and quantitative differences in the composition of the cartilage, to differences in the rate of physiologic development and ageing, and to increased thyroid activity exerting its effect not only directly on the cartilage but also on the metabolism of fat in general.

References

1. Barry, G., & Kennaway, E. L. 1937. The structure of the thyroid in mice of different strains. *Am. J. Canc.*, **29**, 522-529.
2. Bauer, W., & Bennett, G. A. 1936. Experimental and pathological studies in the degenerative type of arthritis. *Journ. Bone and Joint Surg.*, **18**, 1-18.
3. Eddy, W. H., & Dalldorf, G. 1944. The Avitaminoses. The Williams and Wilkins Company, Baltimore.
4. Fenton, P. F., & Cowgill, G. R., with the technical assistance of Stone, M. A. 1947. The nutrition of the mouse. I. A difference in the riboflavin requirements of two highly inbred strains. *Journ. Nutrit.*, **34**, 273-283.
5. Gross, F., & Schwartz, S. 1950. The distribution of thyroxine labeled with radioiodine in C3H and C57 mice. *Cancer Research*, **10**, 222-223.
6. Grüneberg, H. (a) 1943. The genetics of the mouse. Cambridge University press. (b) 1947. Animal genetics in medicine. Paul B. Hoeber, Inc., New York and London.
7. György, P., & Rose, C. S. 1949. The lipotropic effect of estrogenic hormones in inbred rats *Proceed. Soc. Exper. Biol. and Medicine*, **71**, 552-555.
8. Handler, P. F. 1948. The influence of thyroid activity in the liver and plasma lipides of choline-and cystine-deficient rats. *Journ. Biol. Chem.*, **173**, 295-303.
9. Heimer, C. B., Maslow, H. L., & Sobel, A. E. 1949. Influence of thyroid on utilization of vitamin A. *Journ. Nutrit.*, **38**, 325.
10. Johnson, R. M., & Albert, S. 1949. Age and strain differences in phosphorus metabolism in various endocrine organs of mice. *Cancer Research*, **9**, 612.
11. Korteweg, R. 1948. Genetically determined differences in hormone production a possible factor influencing the susceptibility to mammary cancer in mice. *Brit. J. Cancer*, **2**, 91-94.
12. Loeb, L., & Kirtz, M. M. 1939. Effects of transplants of anterior lobes of hypophysis on growth of the mammary gland and on development of mammary gland carcinoma in various strains of mice. *Am. J. Canc.*, **36**, 56-82.
13. ————. 1940-1941. (a) Hormones and the process of ageing. *The Harvey Lectures*, **36**, 228-250. (b) 1945. Cancer and the process of ageing. *Biol. Sympos.*, **11**, 197-216.
14. Maddock, C. L., Wollbach, S. B., & Maddock, S. 1949. Hypervitaminosis A in the dog. *Journ. Nutrit.*, **39**, 117-124.

15. SILBERBERG, M., & SILBERBERG, R. 1943. Influence of the endocrine glands on growth and ageing of the skeleton. *Arch. Pathol.*, **36**, 512-534. 1949/50. Some aspects of the rôle of hormonal and nutritional factors in skeletal growth and development. *Growth*, **13**, 359-368. 1950a. Sex differences in skeletal development of mice fed a high-fat diet. *Federat. Proceed.*, **9**, 343-344. 1950b. Effects of a high-fat diet on the joints of ageing mice. To appear *Archv. Pathol.*, **50**, No. 6.

16. VICARI, E. M. 1943. Adrenal lipids of mice with high and low mammary gland tumor incidences. *Anat. Rec.*, **86**, 523-543.

17. ———, & LITTLE, C. C. 1945. "Lipid-steroid" fractions of mouse adrenal lipids. *Proceed. Soc. Exp. Biol. & Med.*, **58**, 59-60.

Growth, 1950, **14**, 231-249.

THE NATURE OF CYCLE VARIATIONS IN MITOTIC ACTIVITY: THE RELATION OF ALIMENTATION AND NUTRITION TO THIS PHENOMENON*

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Rhythmic phenomena are not uncommon in biological material. In general they may be divided into two categories: (1) those processes which are inherently repetitive at constant intervals, and (2) those which appear to be of this character because of a cyclic pattern of environmental stimulus. In the first group may be placed such manifestations as the rhythmic beat of the heart, the movements of respiration, and the rhythmic character of nervous responses (56). In this group one might also consider certain endocrine functions such as ovulation and menstruation, which are subject to periodic stimulation by the anterior hypophysis. The functions placed in this category are subject only to changes in rate and intensity when alterations occur in the environment; regardless of whether or not the environmental changes are cyclical, the only response which can be elicited is a rhythmic one. In the second group one may place the cellular activities of plants which are dependent upon periodic light stimulation (5, 46, 59, 60, 97, 98), and the sexual activities of some birds, fish and even some mammals which also appear to be largely determined by light (2-6, 7-9, 57). In these instances when the environmental stimulus is not rhythmic, a cyclic response is not elicited.

Daily cyclic variations in the average number of mitoses have been reported in a variety of normal plant and animal tissues since this phenomenon was first observed in the plant, Alluim, by Kellicott (60) and in the kitten by Fortuyn-van Leyden (44, 45). In many of these investigations the emphasis has been placed on light as the

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critical factor determining this periodicity. The rationale of this interpretation in the study of plant material is obvious, as it is also in an interpretation of periodicity of mitotic activity in animal epidermis. It may be further substantiated by the observation of Lewis (62) that light is injurious to tissue cultures. However, the mechanism of action on internal organs is not apparent. In earlier studies Blumenfeld (11, 12) suggested that the temperature to which an organ is exposed may influence the time at which maximum mitotic activity occurs. However, if this were true, then it would be expected that all internal organs exposed to the same temperature would show similar curves of mitotic activity; Blumenfeld later reported that this is not the case (13).

Other reports indicate that the diurnal cycle of mitotic activity is determined by the habits of the animals. Bullough (23, 24) has shown that mitotic activity of the mouse epidermis is related to spontaneous bodily activity; it is lowest during waking hours and at a maximum when the animals are at rest. Artificially produced sleep results in a sharp rise in mitotic activity and forced exercise results in extremely low mitotic activity. In a different type of experiment, we (16) have previously observed that by changing the habitual feeding time of guinea pigs we could correspondingly change the time of daily maximum mitotic activity in the thyroid and adrenal gland; it was suggested that the diurnal cycle of cellular division may be determined by the pattern of availability of cellular nutrients. That undernourishment suppresses mitotic activity has been demonstrated in the thyroid by Rabinovitch (87) and in epidermis by Loeb, Haven, Genther, and Friedman (72).

It might be added that the onset of mitotic activity may not only depend upon the availability of such nutrients but also, in some instances, upon the discharge of certain cellular products. Blumenthal and Loeb (18) have demonstrated this for the adrenal gland in an underfeeding experiment in the guinea pig, and this observation has been substantiated by a number of other investigators (90, 91, 103) with respect to the discharge of lipids following stimulation by the anterior hypophysis. If these factors are important, then the withdrawal of food should alter the pattern of cyclic variations in mitotic activity; this report represents experimental evidence to support such a thesis

Also relevant to this problem is the relation of various dietary ele-

ments to mitotic activity in different tissues. Heilbrunn (54) has stated that the primary factor in determining the number of mitotic divisions which take place in a given interval of time would appear to be the rate of growth of the protoplasm. Normal cells maintain a definite size limit, and growing cells are apparently influenced to divide when that limit is reached. The problem of what determines the rate of mitotic division is therefore essentially a growth problem and may depend primarily upon the amount of food available as well as the rate at which it is taken up. Because of this relation of nutrition to mitotic division and its particular applicability to the present problem we have also taken this opportunity to review the present status of investigations dealing with this subject.

Material and Method

Twenty-seven male guinea pigs, ranging in weight from 250 to 350 grams, were fasted for 24 hours before removal of the thyroid, parathyroid and adrenal glands; water was available throughout the experiment. They were then sacrificed in groups of 4 or 5 at 4-hour intervals during the next 24 hours as shown in Table I. Mitotic counts were carried out on these glands according to methods previously described (17).

TABLE I
The Influence of a 24-Hour Fasting Period on Mitotic Rhythmicity

Time after feeding (in hours)	No. of guinea pigs	Average weight	Mitotic Count Thyroid*	Parathyroid**	Adrenal***
28	4	277 gm.	60.0(60-60)	0.6(0-1)	0.2(0-0.4)
32	4	263 gm.	46.6(20-100)	0.07(0-0.2)	4.6(0.4-10.7)
36	5	306 gm.	36.0(0-60)	0.06(0-0.3)	4.7(2.0-8.8)
40	5	280 gm.	20.0(0-60)	0.18(0-0.5)	11.3(7.8-17.0)
44	5	294 gm.	80.0(20-260)35.0†	0.5(0-1.2)0.33†	15.7(9.6-22.0)
48	4	305 gm.	25.0(0-30)	0	14.5(7.00-24.2)

*Average number of mitoses per thyroid gland (both lobes).
**Average number of mitoses per 10,000 cells.
***Average number of mitoses per section.
Figures in parenthesis represent range of variation in mitotic counts.
†Average when a single high count is omitted.

Results

Thyroid Gland: The mitotic count in normally fed guinea pigs of this age-weight group would be expected to be between 100 and 125 mitoses per thyroid gland, based on observations in a previous study

(19). It is apparent, therefore, that fasting for 28 hours has resulted in a depression in mitotic activity. As this period of non-feeding continues there is a progressive decline in the rate of cell division until an average basal level between 20 and 35 mitoses per gland is reached. One exception to this was found in a guinea pig which showed a mitotic count of 260 and thereby raised the average for the 44-hour group to 80 mitoses per gland. If this one animal is omitted the average for the latter group falls to 35 mitoses per thyroid.

With the exception of the case with a relatively high mitotic count, the histological appearance of all thyroids was essentially similar. The follicles were fairly large and contained hard colloid which showed a tendency to fragment when sectioned. Clusters of phagocytes were occasionally found in the colloid of some follicles. The epithelium was flat for the most part, but in occasional areas, particularly in the specimens removed 28 hours after feeding, low cuboidal epithelium was found. The septal connective tissue while generally loose and areolar, showed a tendency to hyalinize in many areas.

Parathyroid Gland: The results in the parathyroid gland are essentially similar to those found in the thyroid. The count for normally fed male guinea pigs of this age-weight group would be expected to average between 1.0 and 1.2 mitoses per 10,000 cells (19). Only two animals showed counts within this range and one of these was the same guinea pig pointed out as an exception in the section on results in the thyroid gland. If this case is omitted, the average for the 44-hour group falls to 0.33 mitoses per 10,000 cells. The basal level of mitotic activity in the parathyroid appears to be reached slightly earlier than in the thyroid gland (32 hours after feeding as compared with 36 hours) and varies between an average of .07 and .33 mitoses per 10,000 cells.

There is remarkably little variation in the histological pattern of the parathyroid. Masses of small compactly arranged epithelial cells with uniform, dark nuclei are separated into lobules by hyalinized connective tissue.

Adrenal Gland: The results with the adrenal gland are quite different from those observed with the previous two endocrine organs. Again, an average count in normally fed guinea pigs of this age-weight group would be expected to range between 2.4 and 2.6 mitoses per section of adrenal cortex (19). It is obvious therefore that at 28 hours after the last meal there is an extreme depression in mitotic

activity, and this probably represents the basal level. However, there then follows a progressive rise in the number of mitoses through successive periods, reaching a height approximately 6-7 times the normal mitotic count at 44-48 hours after feeding.

In the first two groups (28 and 32 hours after feeding) the zona glomerulosa is thin, consisting of a layer 2-3 cells thick, and the cytoplasm of the cells is granular. The outer two-thirds of the zona fasciculata is fairly thick and many of the cells contain lipid vacuoles in the cytoplasm. The inner fasciculata blends with the zona reticularis and the cells of the latter show an eosinophilic granular cytoplasm. In succeeding groups there occurs a moderate thickening of the glomerulosa, the deeper layers of which tend to blend with the fasciculata as the cells of the latter show progressively fewer lipid vacuoles with longer periods of fasting. There is no remarkable change of the reticularis or of the medulla in any of these animals.

Discussion

It is apparent from these results that the withdrawal of food from these guinea pigs results in an alteration of the diurnal cycle in mitotic activity. In the case of the thyroid and parathyroid gland mitotic activity progressively falls to a basal level, while in the adrenal cortex there is a progressive increase in mitotic activity following a fall to a basal level. The observations in the thyroid gland are in accordance with the findings of Loeb and Hesselberg (74), and of Loeb (68, 69) who have observed that the degree of compensatory hypertrophy of the thyroid of guinea pigs is influenced by the changes in weight which the animals undergo during the period following partial removal of the gland; the hypertrophy is more marked in animals gaining weight during the experiment than in those whose weight remains stationary or decreases. Rabinovitch (87) in continuing these experiments has found, further, that following a period of underfeeding for 10 or 11 days so that the animals lose 20 to 32 per cent of their body weight, the epithelium lining the follicles becomes quite flat and there is an entire absence of mitoses.

Conversely, Rabinovitch and Gray (88) observed that following the administration of optimum doses of iodides which have a specific metabolic relationship to the thyroid, a wave-like reaction in metabolic activity occurs; a period of increase in mitotic activity is followed by a decrease, which in the end may reach below the normal level.

As would be expected from our previous studies with respect to alimentation as well as to iodine stimulation, the results in the parathyroid essentially parallel those in the thyroid (17). The findings in the adrenal cortex are also consistent with previous observations. Blumenthal and Loeb (18) have shown that when guinea pigs are slightly underfed there is a suppression of mitotic activity, while greater degrees of inanition produce an increase in the rate of cell division. Schmeckebier (92) has also observed that underfeeding produces an increase in mitotic activity in the adrenal cortex of guinea pigs. We have correlated this effect with the presence of lipids in the cortical cells, the lipids probably being associated with the adrenal cortical hormones. Cells containing lipid vacuoles seldom undergo mitotic activity, but when there is a marked diminution in the lipid content, proliferative activity becomes most marked. D'Angelo, Gordon and Charipper (38) have confirmed this effect of acute starvation on the adrenal in the guinea pig. In the rat, however, adrenal enlargement was a variable response. Desoxycorticosterone acetate in high doses decreased the size and solid content of the rat adrenal in fed or acutely starved animals, but failed to affect the adrenal size in the fed guinea pig, or to prevent the adrenal hypertrophy of starvation. Recently, Haven, Bloor and Randall (52) noted a hyperplasia of the adrenal in rats with Walker carcinoma 256; this was associated with a decrease in the average per cent of total steroid in the gland. As noted previously, the loss of adrenal cortical lipid occurs also as the initial phase of stimulation of the adrenal cortex by anterior hypophysis hormone when there is a decrease in cholesterol as well as an increase excretion of "cortins" and 17-ketosteroids (90, 91, 103).

These observations in endocrine organs probably obtain also in other organs, either as a direct effect of the altered alimentation or as a secondary effect conditioned by the activity of the endocrine system. Concerning the latter, there are several observations indicating that mitotic activity of the epidermis is influenced by thyroid and ovarian activity. Loeb, Haven, Genther and Friedman (73) have reported results suggesting that thyroid substance and KI cause a lowering in both mitotic activity and the number of cells in the epidermis, while partial thyroidectomy may increase proliferative activity. Loeb and Haven (70, 71) have also shown that there is a relation between functional states of the sex organs of the guinea pig and epidermal

mitotic activity, and Chouke (31, 32) and coworkers have shown a relation between ovarian function and the thyroid gland. In this regard it should also be noted that Loeb (64-67) has observed that undernourishment produces a "hypotypical" state of the ovary. The lack of sufficient food affects first the granulosa cells, which prematurely dissolve; maturation of follicles ceases and the follicles become atretic even before they reach medium size. Loeb, Haven, Genther and Friedman (72) have also noted a direct effect of undernourishment in suppressing mitotic activity of the epidermis, but this requires a considerably longer period of inanition than is necessary to suppress activity of the thyroid or sex organs.

Blumenfeld (13) does not believe that a general influence acting on the entire body can explain maximal mitotic activity in the epidermis at one time, the renal cortex at another, and the submaxillary gland throughout most of the day and night. In a later report (16) he noted a direct relationship between functional activity and diurnal variations in cell division; the rate of mitotic activity in the renal cortex varied inversely with the volume of urine excreted and in the submaxillary gland with the weight of food eaten. Thuringer (102) has noted that a peak in mitotic activity in the epidermis of the cat's paw occurs two hours after mechanical or electrical stimulation. Bullough (23, 24) also observed a correlation between the diurnal rhythm of mitotic activity in the skin, intestinal mucosa and tubules of the epididymis, and bodily activity, but did not find variations in the time of maximum mitotic activity between these different organs. Subsequently, however, Bullough (26) was able to correlate mitotic activity and glucose metabolism, and recently (27) he observed that starvation has a powerful effect in depressing epidermal mitotic activity in mice, the latter results being in substantial agreement with certain findings in the present report. The apparent relation between bodily activity and rate of mitotic division is not incompatible with the concept that the entrance of nutritional elements into the cell plays a rôle in the initiation of mitotic activity since, in general, as Wilson (104) has pointed out, feeding and bodily activity go together.

A relation between mitotic activity in the liver and alimentation is more evident than between feeding habits and the activity of other body organ systems. Yet our investigations as well as those of others show this to be true of organs other than those directly involved in the process of alimentation. It is not necessary to assume

that nutritional constituents must act simultaneously on all organs, since one organ may synthesize substances from nutritional elements available to it as an initial step in further synthesis or utilization by another organ. Anabolic and catabolic processes which may influence mitotic activity may be in different phases in different organs at any given time.

These observations, therefore, indicate an intimate relationship between the rhythm of alimentation and ensuing metabolic processes on the one hand, and mitotic activity on the other. Any alteration in the pattern of the former may result in changes in the temporal pattern of the latter. Starvation depresses mitotic activity and eliminates cyclic variations in most organs, but in some with special metabolic functions it may produce a marked increase in the number of mitoses during certain phases of starvation.

Cyclic variations have now been observed in normal and regenerating epidermis (11, 12, 15, 21, 23, 24, 29, 34, 36), salivary gland (13), renal cortex (10, 12), thyroid (16), adrenal cortex (16), epididymis, esophagus and duodenal mucosa (23); it appears to be absent (at least on a 24-hour basis) in intestinal lymphoid tissue (23), semi-niferous tubules (23) and malignant tumors (13, 40) in all of which a high rate of mitotic activity is almost constantly present. However, even in these instances the rate of mitotic activity may depend upon the nutritional state of the animal. Starvation and dietary restrictions may decrease the growth rate of tumors and lower the incidence of spontaneous neoplasms (75, 89, 99, 100).

The division of rhythmic biological phenomena into two groups as noted in the introduction is not always a distinct one. Gerard (48) states that regular rhythmic action can occur as a result of constant and unvarying forces and cites as an example the continuous activity of some endocrine glands which underlies the rhythmic changes of ovulation and menstruation. It might therefore be assumed that the latter functions are of the first type noted in the introduction, but even such rhythms can be modified in some species by certain extraneous factors such as light; Benoit (2-4, 22) and others have shown that this appears to be due to a reflex stimulation of the anterior hypophysis. Conversely, cyclic mitotic variations in plants are thought to be related to the periodicity of light exposure, but Sulkin (98) has observed that in the root tip of Crotalaria retusa diurnal variations in the number of cell divisions are independent of light

and temperature. Nevertheless, Freisner (46) concluded from a study of a variety of seedlings and roots that the nature of the mitotic curve depends not so much on the time of day as on the time of initiation of metabolic activity; periodicity in plants is therefore probably of the second type. It thus becomes important to distinguish between factors which may modify a basic rhythm and those which are responsible for the cyclic pattern. It is equally important to distinguish between bodily activities which may be an apparent factor and the rhythm of alimentation which is probably the basic factor and which may be modified by altering the pattern of bodily activities. Cyclic variations in mitotic activity are, therefore, in all likelihood, of the second type.

An understanding of the mechanism of mitotic rhythm as well as the processes generally initiating mitotic activity depends finally on an understanding of the steady cell metabolism. It is apparent that the initiation of mitotic activity in complex organisms cannot be attributed to any single extracellular substance, since a physiological state which may bring about a depression in mitotic activity in the cells of thyroid and parathyroid glands may result in a marked increase in proliferative activity in the adrenal cortex. Furthermore, certain substances may act as mitotic stimulants on specific cells. As pointed out previously, iodine has such an effect on the epithelium of the thyroid follicle and para aminobenzoic acid may produce a similar histological effect (30, 50). Conversely, vitamin A (78) and foods containing nitriles suppress mitotic activity in the thyroid (1, 76). Pantothenic acid and probably also riboflavin appear necessary for adrenal cortical function in the rat (37) and ascorbic acid is essential for normal adrenal function in those species which are unable to synthesize this vitamin. Drill (39) has shown that deficiencies in diet may particularly suppress the output of the specific trophic hormones of the pituitary and thus suppress not only the other endocrine organs, but also general body growth processes.

In an analysis of the elements in the diet which may specifically affect mitotic activity, studies on the effects of deprivation or of administration of excessive amounts of certain food elements are applicable here only to a limited extent, since most of these have been carried out for periods considerably longer than 24-48 hours. Some experimental observations may relate to the rôle of proteins, fats and carbohydrates in regulating relatively short, periodic growth

responses. Wilson and Leduc (105) have observed that boiled and autolyzed liver, pulped kidney and boiled egg yolk injected intraperitoneally at first inhibit mitosis for 3-6 days, following which there is a sharp rise in mitotic activity in the liver of young mice; in adult mice this treatment results in a wave of mitotic activity usually within three days after the injection of these substances. The presence of a latent period suggests that these substances may have to be converted to some other form before they are able to act as mitotic stimulants. It is worthy of note that the curves obtained by Wilson and Leduc parallel closely curves of hemolysin production in the rat after the administration of protein-containing substances (Cannon 28), thus further suggesting a relation between mitotic activity and protein utilization.

Specifically with regard to protein, Leduc (61) has shown that mitotic activity in the liver of young weaning mice disappears during fasting and reappears after subsequent refeeding. The amount of protein in the diet which is refed determines the time of appearance of the peak of mitotic activity. In adult mice in which no mitotic activity ordinarily occurs in the liver, fasting and subsequent refeeding initiates mitotic activity. Silberberg and Silberberg (95) have noted that in mice fed a high casein diet, growth and development of the epiphyseal cartilage is accelerated. In certain organs specific amino acids may be related to growth responses. Maun, Cahill and Davis (77) have observed a narrowing of the epiphyseal cartilage of the long bones, with atrophy of the thymus, adrenal cortex and testicles, in rats deficient in phenylalanine or leucine. While no histological observations have been made relative to the rapidity of changes in mitotic activity in animals placed on diets deficient in essential amino acids, the changes in nitrogen balance are sufficiently rapid to indicate a possible relation to the present study. Further evidence of a rapid onset of utilization of dietary proteins is presented by Henderson and Harris (55) who have noted that "a delay of 3 hours or longer in the feeding of lysine as a supplement to a lysine low diet interfered with metabolism."

The relation of carbohydrates and related substances to the diurnal cycle has been studied by Wilson (104). The cycle in the liver of young mice has been correlated with the glycogen cycle in the hepatic cell; mitotic activity is highest in the morning, with a drop in the late afternoon and early evening, while glycogen storage is

high during the night, with a falling off during the late morning to a low in the afternoon and early evening. As noted above, Bullough (25) has recently observed that epidermal mitotic activity in the adult mouse is strongly influenced by the concentration of sugar or glycogen in the tissues. A high rate of mitotic activity occurs when blood sugar is deposited in the tissues during sleep, and it can be raised artificially by the injection of carbohydrate. Conversely, a reduction in the blood sugar level produced with insulin, or the administration of phlorizin which interferes with the phosphorylation of sugar, results in an almost complete loss of epidermal mitotic activity. The phase of hyperplasia of the adrenal cortex in the present experiments may possibly represent the effect of a hypoglycemic state, which results in the initiation of glyconeogenesis, a process requiring an increased output of adrenal cortical hormone. Potter, Schneider and Liebl (82) have observed that succinic dehydrogenase, cytochrome oxidase and adenosine triphosphatase increase markedly in rat brain and liver during a relatively short period of 10-15 days after birth, at which time the adult level is approximated. This corresponds closely to the period in which we have noted a relatively high degree of mitotic activity in the thyroid, parathyroid and adrenal cortex of guinea pigs (19). At about the time there is a levelling off in the concentration of these enzymes there occurs a sharp drop in proliferative activity in these glands.

It might be expected that certain fatty or lipid substances would influence the rate of cell division because of their known effects in altering the permeability of cell membranes. Furthermore, Heilbrunn (54) states that fat solvents prevent protoplasmic gelation which appears to be necessary for spindle formation. Nevertheless, little is reported of possible specific effects of these dietary elements on mitotic division. Silberberg and Silberberg (94, 96) have noted that a high fat diet markedly hastens skeletal growth and development in mice. Whether these changes are specifically due to the fat content of the diet or to the high caloric intake has not as yet been reported. Certain studies in tumors are also relevant. Boutwell, Brush and Rusch (20), as well as Tannenbaum and Silverstone (101), have demonstrated a stimulating effect of fat on the rate of formation of certain types of tumors. Tannenbaum believes this effect to be related to the proportion of fat in the diet, while Rusch and coworkers attribute it to the net energy effect of the dietary fat content. It

appears, however, that the type of fat is also important. Miller and associates (79) have shown that a protective effect against the formation of tumors of the liver by azo dyes can be obtained by replacing the corn oil in the diet by hydrogenated coconut oil or its chief constituent, lauric acid. Other data which may be taken as evidence that lipids may inhibit mitotic activity are those relating to the discharge of adrenal cortical lipids as a first phase of hyperplasia of the adrenal cortex as noted previously.

In general, mitotic activity is probably related to intracellular enzyme activity, hormones and other similar specific substances may influence the activity of or take part in such enzyme systems. For example, Price, Colowick and Cori (84) have demonstrated the influence of insulin, anterior pituitary and adrenal-cortical hormones on the activity of the enzyme hexokinase. It is possible that the initiation of cell division depends upon the activation of certain enzyme systems and the inhibition of others. In studies on the chick embryo, Moog (80) has observed that in certain stages of development, the alar plate of the spinal cord shows marked mitotic activity and is almost devoid of alkaline phosphatase activity, while the basal plate contains a high concentration of this enzyme and a low degree of proliferative activity. Hamburger (51) has observed a wave-like increase in mitotic activity in the spinal cord of the chick embryo, which ends at about 8½ days of incubation. His results indicate that, in all likelihood, the agents which activate cell division reside in the neural epithelium itself, since those areas showing marked mitotic activity are exposed to the same circulatory mechanisms as areas showing low mitotic activity. Since Hamburger's studies were made on nearby portions of spinal cord, it would be expected that they would act in a similar manner to extracellular influences. In the embryo the nature of such intracellular agents is unknown, although Eakin (41) has recently presented evidence supporting the theory that specific inductors as well as non-specific stimuli may exert their effect by setting free from a bound state, or activating a self-duplicating X substance which then controls subsequent differential growth; such a substance is probably in the nature of an enzyme. The apparent increase in activity of enzymes related to carbohydrate metabolism in actively proliferating tissues may represent the activation of intracellular substances initiating mitotic activity, but it is also possible that carbohydrates and certain other food elements have a rela-

tion to mitotic activity only in that they furnish a source of energy for this process. In the latter instance it would be expected that the carbohydrates would play a predominant rôle, since they represent the most readily available source of energy. In this connection Goldinger and Barron (9) have observed that an increased utilization of glycogen accompanied by an increased utilization of pyruvate may provide the energy necessary for cell division. A further possibility would explain on a single basis the apparently independent ability of carbohydrates and proteins to act as mitotic stimulants. Besides participating in protein synthesis, amino acids are used for the formation of enzymes, hormones and vitamins; the latter two types of substances probably also form essential parts of enzyme systems. Carbohydrates and fats contribute to the formation of non-essential amino acids which may be utilized in the intracellular formation of enzymes. Furthermore, the nitrogen-sparing action of carbohydrates may allow for the utilization of protein which would not otherwise be made available for enzyme formation. The interrelation of the various food elements in protein metabolism has recently been reviewed by Geiger (47). The correlation of the quantity of certain intracellular enzymes (Potter, Schneider and Liebl, 82) with mitotic activity (Blumenthal, 19) during early post-natal development would tend to substantiate the concept that mitosis occurs in relation to intracellular enzyme production.

Data are available with which it may be possible to localize to some extent, the sites of such enzyme activity. Price, Miller, Miller and Weber (85) have recently reported that there is an increase in the nuclear fraction of protein and desoxypentose nucleic acid of the liver of rats fed azo dyes for one month (before carcinoma develops) which seems to be related to bile duct proliferation, whereas a decrease in these elements which occurs in the large granular fraction is probably due to changes in the hepatic parenchymal cells in which there is a loss of mitochondria. Associated also with these histological observations there is a decrease in succinoxidase content of the liver and of the mass of mitochondria (Potter, Price, Miller and Miller, 83). It is well to compare these findings with certain older observations of Jacques Loeb (63) who noted that during the first period of division of the amphibian egg, the nuclear material increases in a manner which indicates that cytoplasmic materials are transformed into nuclear substance. Nuclear division may occur at fairly regular inter-

vals, and at each division the nuclear material is approximately doubled. He further observed that the mass of nuclear material produced at each division is proportional to the mass already present, and concluded that the reaction which produces nuclear material is catalyzed by some constituent of the nucleus, so that the greater the amount of nuclear material already on hand, the more rapid the reaction or the more catalyst present to convert cytoplasm to nuclear material. This might account for the loss of mitochondria and the decrease in certain cytoplasmic enzymes as well as the increase in intranuclear proteins in actively proliferating tissues. Recent advances in histochemical techniques may offer a tool for the further analysis of the problem of enzyme activity in its relation to the process of mitosis, particularly the possible movement of enzymes from cytoplasm to nucleus, as an initial part of the process of mitotic division.

Summary

Periodic mitotic counts beginning 28 hours after the last feeding were carried out in the thyroid, parathyroid, and adrenal glands of guinea pigs. There was a loss of mitotic periodicity in all three endocrine organs. The thyroid and parathyroid glands showed a progressive diminution in mitotic activity over the 24-hour period during which counts were made, while the adrenal cortex showed an initial period of depression followed by an increase in proliferative activity.

These results along with those of a previous report show that mitotic periodicity may be a function of the cyclic pattern of alimentation. The relation of the intracellular enzyme-systems and the availability of substrate to the initiation of mitotic activity are discussed.

References

1. Astwood, E. B. 1943. The chemical nature of compounds which inhibit the function of the thyroid gland. *J. Pharmacol. & Exp. Therap.* **78**, 79-89.
2. Benoit, J. 1935. Influence de a lumiére naturelle sur la croissance testiculaire chez le canard au cours réprise sexuelle saisonniére. *Compt. rend. Soc. de biol.* **120**, 131-133
3. ———. 1935. Stimulation par la lumiére artificielle du developpement testiculaire chez des canards aveuglés par section du nerf optique. *Compt. rend. Soc. de biol.* **120**, 133-136.
4. ———. 1935. Stimulation par la lumiére artificielle du developpement testiculaire chez des canards aveuglés par enucleation des globes oculaire. *Compt. rend. Soc. de biol.* **120**, 136-139.

5. BERINSOHN, H. W. 1919. The influence of light on the cell increase in the roots of Alluim Cepa. Verhandl. Kon. Akad. van Wetenschapp., Amsterdam.

6. BISSONETTE, T. H. 1931 Studies on the sexual cycle in birds. IV. Experimental modification of the sexual cycle in males of the European Starling (Sturnus vulgaris) by changes in the daily period of illumination and of muscular work. *J. Exp. Zoöl.* **58**, 281-319.

7. ————. 1931. Studies on the sexual cycle in birds. V. Effect of light of different intensities upon the testis activity of the European Starling (Sturnus vulgaris). *Physiol. Zoöl.* **4**, 542-574.

8. ————. 1933. Light and sexual cycles in starlings and ferrets. *Quart. Rev. Biol.* **8**, 201-208.

9. ————, & CSECH, A. G. 1939. Modified sexual photoperiodicity in cottontail rabbits. *Biol. Bull.* **77**, 364-367.

10. BLUMENFELD, C. M. 1938. Periodic and rhythmic mitotic activity in the kidney of the albino rat. *Anat. Rec.* **72**, 435-443.

11. ————. 1939. Periodic mitotic activity in the epidermis of the albino rat. *Science*, **90**, 446-447.

12. ————. 1942. Normal and abnormal activity. I. Comparison of periodic mitotic activity in epidermis, renal cortex and submaxillary salivary gland of the albino rat. *Arch. Path.* **33**, 770-776.

13. ————. 1943. Studies of normal and of abnormal mitotic activity; rate and periodicity of mitotic activity of experimental epidermoid carcinoma in mice. *Arch. Path.* **35**, 667-673.

14 ————. 1943. Studies of normal and of abnormal mitotic activity; rate and periodicity of mitotic activity in regenerating epidermis of healing wounds in rabbits. *Arch. Path.* **36**, 493-498.

15. ————. 1944. Studies on Cancer: 1. The relatonship of function, light and temperature to growth by mitosis. *Arch. Path.* **38**, 321-325.

16. BLUMENTHAL, H. T. 1940. The influence of time of feeding on the periodicity in mitotic activity in thyroid and adrenal gland of normal male guinea pigs. *Endocrinol.* **27**, 481-485.

17. ————. 1942. Influence of weight (age), diet and dosage on response of thyroid and parathyroid glands of male guinea pig to potassium iodide; effect of this substance on adrenal. *Endocrinol.* **31**, 226-236.

18. ————, & LOEB, L. 1942. Two antagonistic effects of underfeeding on the adrenal cortex of the guinea pig. *Am. J. Path.* **18**, 615-631.

19. ————. 1945. Ageing processes in the endocrine glands of the guinea pig. *Arch. Path.* **40**, 264-269.

20. BOUTWELL, R. K., BRUSH, M. K., & RUSCH, H. P. 1949. The stimulating effect of dietary fat on carcinogenesis. *Canc. Res.* **9**, 741-746.

21. BRODERS, A. C., & DUBLIN, W. B. 1939. Rhythmicity of mitosis in the epidermis of human beings. *Proc. Staff Meetings, Mayo Clinic* **14**, 423-425.

22. BROWMAN, L. G. 1937. Light in its relation to activity and estrus rhythms in the albino rat. *J. Exper. Zoöl.* **75**, 375-388.

23. BULLOUGH, W. S. 1948. Mitotic activity in the adult male mouse, Mus Musculus L. The diurnal cycles and their relation to waking and sleeping. *Proc. Royal Soc. B.* **135**, 212-232.

24. ————. 1948. The effects of experimentally induced rest and exercise on the epidermal mitotic activity of the adult male mouse, Mus Musculus L. *Proc. Royal Soc. B.* **135**, 233-242.

25. ————. 1949. The relation between epidermal mitotic activity and the blood sugar level of the mouse, Mus Musculus L. *J. Exp. Biol.* **26**, 83-99.

26. ————. 1949. The effects of high and low temperature on the epidermal mitotic activity of the adult mouse, Mus Musculus L. *J. Exp. Biol.* **26**, 76-82.

27. ————. 1949. The effect of a restricted diet on mitotic activity in the mouse. *Brit. J. Canc.* **3**, 275-277.

28. CANNON, P. R. 1948. Protein and amino acid deficiencies. C. C. Thomas, Springfield, Ill.

29. CARLETON, A. 1934. A rhythmical periodicity in the mitotic division of animal cells. *J. Anat.* **68**, 251-263.

30. CHESNEY, A. M., CLAWSON, T. A., & WEBSTER, B. 1928. Endemic goitre in rabbits. I. Incidence and characteristics. *Bull. John Hopkins Hosp.* **43**, 261-277.

31. CHOUKE, K. S., FRIEDMAN, H., & LOEB, L. 1935. Proliferative activity of the thyroid gland during the sexual cycle. *Anat. Rec.* **63**, 131-137.

32. ————, & BLUMENTHAL, H. T. 1942. Further investigations on the proliferative activity of the thyroid gland of the female guinea pig during the sexual cycle. *Endocrinol.* **30**, 511-515.

33. CLARK, L. B., LEONARD, S. L., & BUMP, G. 1937. Light and the sexual cycle of game birds. *Science* **85**, 339-340.

34. COOPER, Z. K. 1939. Mitotic rhythm in human epidermis; introduction and review of literature. *J. Invest. Dermat.* **2**, 289-300.

35. ————, & FRANKLIN, H. C. 1940. Mitotic rhythm in epidermis of the mouse. *Anat. Rec.* **78**, 1-8.

36. ————, & SCHIFF, A. 1938. Mitotic rhythm in human epidermis. *Proc. Soc. Exp. Biol. & Med.* **39**, 323-324.

37. DAFT, F. S., & SEBRELL, W. H. 1939. Hemorrhagic adrenal necrosis in rats on deficient diets. *Pub. Health Rep.* **54**, 2247-2256.

38. D'ANGELO, S. A., GORDON, A. S., & CHARIPPER, H. A. 1948. A differential response of the rodent adrenal gland to acute starvation. *Proc. Soc. Exp. Biol. & Med.* **68**, 527-529.

39. DRILL, V. A. 1943. Interrelations between thyroid function and vitamin metabolism. *Physiol. Rev.*, **23**, 355-379.

40. DUBLIN, W. B., GREGG, R. O., & BRODERS, A. C. 1940. Mitosis in specimens removed during day and night from carcinoma of the large intestine. *Arch. Path.* **30**, 893-895.

41. EAKIN, R. M. 1949. The nature of the organizer. *Science*, **109**, 195-197.

42. EUFINGER, H., & GOTTLIEB, J. 1933. Wirkungskoeffizienten des thyroxin-effektes in biologischen Versuch. *Klin. Wchnschr.* **12**, 1397-1402.

43. FISKE, V. M. 1939. Effects of light and darkness on the activity of the pituitary of the rat. *Proc. Soc. Exp. Biol. & Med.* **40**, 189-191.

44. FORTUYN-VAN LEYDEN, C. E. D. 1917. Some observations on periodic nuclear divisions in the cat. *Proc. Akad. wet. Amsterdam* **19**, 38-44.

45. ————. 1926. Day and night period in nuclear divisions. *Proc. Akad. wet.* Amsterdam **29**, 979-988.

46. FREISNER, R. C. 1920. Daily rhythm of elongation and cell division in certain roots. *Am. J. Botany* **7**, 380-407.

47. GEIGER, E. 1950. The rôle of the time factor in protein synthesis. *Science* **111**, 594-599.

48. GERARD, R. W. 1940. Unresting cells. Harper Bros. and Co. New York, N. Y.

49. GOLDINGER, J. M., BARRON, E. S. G. 1946. The pyruvate metabolism of sea urchin eggs during cell division. *J. Gen. Physiol.* **30**, 73-82.

50. GORDON, A. S., GOLDSMITH, E. D., & CHARIPPER, H. A. 1945. Effects of para aminobenzoic acid and thiouracil on thyroid function and resistance to low pressure. *Endocrinol.* **37**, 223-229.

51. HAMBURGER, VIKTOR. 1948. The mitotic patterns in the spinal cord of the chick embryo and their relation to histogenetic processes. *J. Comp. Neur.* **88**, 221-284.

52. HAVEN, F. L., BLOOR, W. R., & RANDALL, C. 1949. Lipids of the carcass, bood plasma and adrenals of the rat in cancer. *Canc. Res.* **9**, 511-514.

53. HERCUS, C. E., & PURVES, H. D. 1936. Studies on endemic and experimental goiter. *J. Hyg.* **36**, 182-203

54 HEILBRUNN, L. V. 1943. An outline of general physiology, W. B. Saunders, Phila. Second Edition, 1943.

55. HENDERSON, R., & HARRIS, R. S. 1949. Concurrent feeding of amino acids. *Fed. Proc.* **8**, 385.

56. HOAGLAND, H. 1949. Rhythmic behavior of the nervous system. *Science,* **109**, 157-164.

57. HOOVER, E. E. 1937. Experimental modification of the sexual cycle in trout by control of light. *Science* **86**, 425-426.

58. JENSEN, H., & GRATTEN, J. F. 1940. Identity of glycotropic (anti-insulin) substance of anterior pituitary gland. *Am. J. Physiol.* **128**, 270-275.

59. KARSTEN, B. 1918. Uber die Tagesperiode der Kern-u Zellteilungen. *Ztschr. f. Bot.* **10**, 1-20.

60. KELLICOTT, W. E. 1904. The daily periodicity of cell division and of elongation in the root allium. *Bull. Torrey Bot. Club* **31**, 529-550.

61. LEDUC, E. H. 1949. Mitotic activity in the liver of the mouse during inanition followed by refeeding with different levels of protein. *Am. J. Anat.* **84**, 397-430.

62. LEWIS, M. R. 1935. Photosensitivity of chick-embryo cells growing in media containing certain carcinogenic substances. *Am. J. Canc.* **25**, 305-309.

63. LOEB, J. 1913. Artificial parthenogenesis and fertilization. U. of Chicago Press, Chicago, Ill.

64. LOEB, L. 1911. Ueber Hytopie des zyklischen Veraenderungen des saeugetiere ovariums und ueber ihre Beziehung zur Sterilitaet. *Zentralbl. f. Physiol.* **25**, 342-343.

65. ———. 1917. The concrescence of follicles in the hypotypical ovary. *Biol. Bull.* **33**, 187-195.

66. ———. 1917. Factors in the growth and sterility of the mammalian ovary. *Science* **45**, 591-592.

67. ———. 1921. Effect of undernourishment on mammalian ovary and the sexual cycle. *J.A.M.A.* **77**, 1646-1648.

68. ———. 1929. Structural changes which take place in thyroid glands of guinea pigs during the process of compensatory hypertrophy under the influence of iodine administration. *Endocrinol.* **13**, 49-62.

69. ———. 1929. Studies on compensatory hypertrophy of thyroid gland; a comparison between the effect of administration of thyroxin, thyroid and anterior pituitary substance on compensatory hypertrophy of the thyroid gland in the guinea pig. *Am. J. Path.* **5**, 71-78.

70. ———, & HAVEN, F. L. 1927. Effect of cyclic changes in the female guinea pig on cell proliferation in epidermis. *Proc. Soc. Exp. Biol. & Med.* **24**, 898-900.

71. ———. 1929. The relation between functional states of the sex organs in the female guinea pig and the cell proliferation in the epidermis. *Anat. Rec.* **43**, 1-26.

72. ———, GENTHER, I. T., & FRIEDMAN, H. 1930. The effect of undernourishment on the proliferative activity and structure of the epidermis of the guinea pig ear. *Anat. Rec.* **46**, 55-64.

73. ————, & FRIEDMAN, H. 1930. The effect of administration of thyroid substance, of potassium iodide and of extirpation of the greater portion of the thyroid gland on the epidermis of the guinea pig. *Anat. Rec.* **46**, 65-80.

74. ————, & HESSELBERG, C. 1920. III. Studies on compensatory hypertrophy of the thyroid gland. B. Change in weight in the host as a factor in compensatory hypertrophy. *J. Med. Res.* **41**, 295-298.

75. ————, SUNTZEFF, V., BLUMENTHAL, H. T., & KIRTZ, M. M. 1942. Effect of weight on the development of mammary carcinoma in various strains of mice. *Arch. Path.* **33**, 845-865.

76. MARINE, D. E., BAUMAN, E. J., SPENCE, A. W., & CIPRAX, A. 1932. Further studies on the etiology of goiter with particular references to the action of cyanides. *Proc. Soc. Exp. Biol. & Med.* **29**, 772-775.

77. MAUN, M. E., CAHILL, W. M., & DAVIS, R. M. 1945. Morphologic studies of rats deprived of essential amino acids. I. Phenylalanine. *Arch. Path.* **39**, 294-300. II. Leucine, *Arch. Path.* **40**, 173-178. III. Histidine, *Arch. Path.* **41**, 25-31.

78. MCCARRISON, R. 1923. Pathogenesis of deficiency disease. XI. Observations on fat excess in relation to iodine requirements and to the thyroid gland. *Ind. J. Med. Res.* **11**, 1-51.

79. MILLER, J. A. 1947. Studies on the mechanism of the effects of fats and other dietary factors on carcinogenesis by the azo dyes. *Ann. N. Y. Acad. Sci.* **49**, 19-28.

80. MOOG, F. 1943. The distribution of phosphatase in the spinal cord of chick embryos of one to eight days incubation. *Proc. Nat. Acad. Sci.* **29**, 176-183.

81. ORTIZ PICON, J. M. 1933. Über Zellteilungsfrequenz und Zellteilungsrhythmus in der epidermis der Maus. *Ztschr. f. Zellforsch. u. mikr. Anat.* **23**, 779-789.

82. POTTER, V. R., SCHNEIDER, W. C., & LEIBL, G. J. 1945. Enzyme changes during growth and differentiation in the tissues of the newborn rat. *Canc. Res.* **5**, 21-24.

83. ————, PRICE, J. M., MILLER, E. C., & MILLER, J. A. 1950. Studies on the intracellular composition of livers from rats fed various aminoazo dyes. III. Effects on succinoxidase and oxalacetic oxidase. *Canc. Res.* **10**, 28-35.

84. PRICE, W. H., CORI, C. F., & COLOWICK, S. P. 1945. Effect of anterior pituitary extract and of insulin on hexokinase reaction. *J. Biol. Chem.* **160**, 633-634.

85. PRICE, J. M., MILLER, E. C., MILLER, J. A., & WEBER, G. M. 1950. Studies on the intracellular composition of livers from rats fed various aminoazo dyes. II. 3′-methyl-, 2′ methyl, and 2-methyl-4-dimethylaminoazobenzene, 3 methyl-4-monomethylaminoazobenzene, and 4′-fluoro-4-dimethylaminoazobenzene. *Canc. Res.* **10**, 18-27.

86. RALLI, E. P., & GRAEF, I. 1945. The effects of the synthetic and natural hormones of the adrenal cortex on melanin deposition in adrenalectomized black rats fed diets adequate and deficient in the filtrate factors of vitamin B_1. *Endocrinol.* **37**, 252-261.

87. RABINOVITCH, J. 1929. The effect of underfeeding on the proliferative activity of the thyroid gland in the guinea pig. *Am. J. Path.* **5**, 87-90.

88. ————, & GRAY, S. H. 1930. The effect of potassium iodide upon the thyroid gland of underfed guinea pigs. *Am. J. Path.* **6**, 75-77.

89. SAXTON, J. A., SPERLING, G. A., BARNES, L. L., & MCCAY, C. M. 1948. The influence of nutrition upon the incidence of spontaneous tumors of the albino rat. *Acta de l'Union Scand. contre le Canc.* **6**, 423-431.

90. SAYERS, G., SAYERS, M. A., WHITE, A., & LONG, C. N. H. 1943. Effect of pituitary adrenotropic hormone on cholesterol content of the rat adrenal glands. *Proc. Soc. Exp. Biol. & Med.* **52**, 200-202.

91. ————, Liang, T., & Long, C. N. H. 1946. Effect of pituitary adrenotrophic hormone on cholesterol and ascorbic acid content of the adrenal of the rat and guinea pig. *Endocrinol.* **38**, 1-9.

92. Schmeckebier, M. M. 1934. Experimentally modified mitotic activity of the adrenal gland in the guinea pig. *Proc. Soc. Exp. Biol. & Med.* **31**, 770-772.

93. Sharpless, G. R., Pearsons, J., & Prato, G. 1939. Production of goiter in rats with raw and with treated soy bean flour. *J. Nutrition* **17**, 545-555.

94. Silberberg, M., & Silberberg, R. 1949. Some aspects of the rôle of hormonal and nutritional factors in skeletal growth and development. *Growth* **13**, 359-368.

95 ————. 1949. Skeletal growth and development in mice fed a high protein diet. *Arch. Path.* **48**, 331-341.

96. ————. 1950. Skeletal growth and articular changes in mice receiving a high fat diet. *Am. J. Path.* **26**, 113-131.

97. Stalfelt, M. G. 1921. Stud. über die Periodizitat d. Zellteilung u. sich daran anschliessende Erscheinungen. *Kungl. Svenska Vetensk. Hand.* **62**, 4-113.

98. Sulkin, N. M. 1938. Cytological studies on Crotalaria retusa with special reference to the chromenemata cycle. U. of Alabama Studies.

99. Tannenbaum, A. 1940. Initiation and growth of tumors. I. Effects of underfeeding. *Am. J. Canc.* **38**, 335-350.

100. ————. 1947. Effects of varying caloric intake upon tumor incidence and tumor growth. *Ann. N. Y. Acad. Sci.* **49**, 5-17.

101. ————, & Silverstone, H. 1949. Dependence of formation of spontaneous mammary carcinoma in mice on the proportion of dietary fat. *Canc. Res.* **9**, 607-608.

102. Thuringer, J. M. 1939. Mitotic index of palmar and plantar epidermis in response to stimulation. *J. Invest. Dermatol.* **2**, 313-326.

103. Tyslowitz, A., & Astwood, E. B. 1942. Influence of pituitary and adrenal cortex on resistance to low environmental temperatures. *Am. J. Physiol.* **136**, 22-31.

104. Wilson, J. W. Personal Communication.*

105. ————, & Leduc, E. M. 1947. Mitotic rate in mouse liver following the intraperitoneal injection of liver, kidney and egg yolk. *Anat. Rec.* **97**, 470-494.

*We wish to thank Dr. J. W. Wilson for making available to us his unpublished data on diurnal rhythm of mitotic activity in the liver of the mouse which was presented at the Centennial Meetings of the A. A. A. S. in Washington, D. C., Sept. 13-17, 1948.

*We also wish to express our appreciation for the numerous suggestions offered by Dr. Leo Loeb in the preparation of this manuscript.

Growth, 1950, **14**, 251-252.

The Methods of Tissue Culture, Parker, Raymond C., 2nd edition, revised, 294 pp., 113 Illus. Paul B. Hoeber, Inc., New York, 1950.

REVIEWED BY PHILIP R. WHITE

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The first edition of Dr. Parker's book, published in 1938 filled a very real need and immediately became a standard reference. The present edition, rather extensively revised, maintains the former high level of excellence in its text and in the illustrations, which are indeed superb. Both the author and the publishers are to be congratulated on an excellent job.

Nevertheless, the book has bothered me. Some faults were easy to identify. It still vaguely annoys me to find a book bearing the title of "Methods of Tissue Culture" making no mention of the culture of plant tissues. Twelve years ago that was excusable though annoying. Today it is hardly excusable. It is disconcerting to find only half a page on the cleaning of general glassware, by only one method, that with white soap. Not that Hanks' sodium meta-silicate method, Earle's H_2SO_4-HNO_3 method, Gey's pyrophosphate method, the various detergent methods, the standard H_2SO_4-$K_2Cr_2O_7$ method, and others are certainly superior, but surely they at least deserve mention! Most people, by the time they are ready to attempt tissue cultures know a bit about measuring pH and do not need to be told in detail. Not one laboratory out of ten will ever have any occasion to do cryoscopic measurements of osmotic value. It was surprising to find no mention of bleeding chickens from the heart, a method which gives far more uniform results than the much more complicated method of bleeding from the carotid. On the other hand, I found the discussions on sterilization of equipment and fluids excellent and the chapters on hanging drop cultures and flask cultures very fine.

But these details, good or bad as they may be still left me puzzled until finally it dawned on me what was the matter. The discursiveness which added so much to the interest and readability of the earlier

edition has in the revision been accentuated. The book is no longer a handbook for the use of students and workers, to acquaint them with all the facts they will require in order to do good tissue cultures, unencumbered by extraneous facts. It has become what in the earlier edition it was in part, but to a much less noticeable degree, a beautifully done but rather rambling survey of the methods and the problems and the work which has interested Dr. Parker. He is interested in the cultivation of viruses in tissue cultures so on page 29 he tells us that in the work of Lloyd, Theiler and Ricci, "The cultures were incubated at 37° C. and subcultures were made every three or four days. After cultivation through eighteen subcultures in this medium, a separate subline was initiated in a medium containing minced whole chick embryo. After further cultivation through fifty-eight subcultures in the latter medium . . ." etc. for a full half page. Very interesting, but how does it help the general tissue culturist, the person wanting to cultivate viruses, or even the person wishing to cultivate this particular virus? What place have these particular details in a book on "Methods of Tissue Culture"?

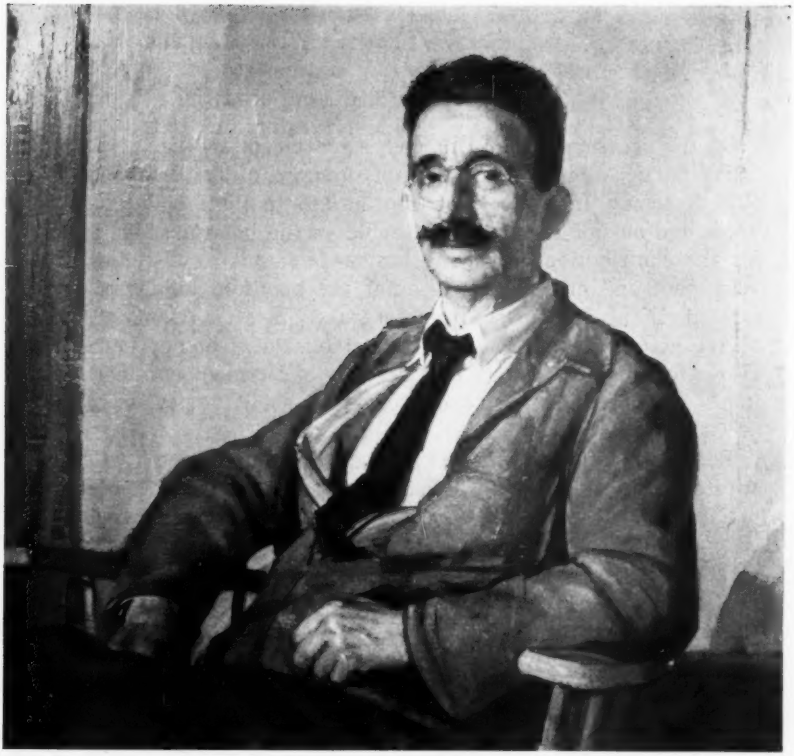
I am sure that everyone who does or wishes to do tissue cultures, unless he is very impatient (in which case he can *never* be a tissue culturist), will want to own this book, to pick it up and browse in it and enjoy it, but I suspect that he will do so far more in his easy chair than at his work table.

On the occasion of Dr. Hammett's retirement in June, 1950, from active participation in the research program of the Lankenau Hospital Research Institute, it is appropriate that his colleagues, both inside and outside the Institute, should join in honoring him, not only for the contributions which he has made in his own studies of development and growth, but also for the manifold influences he has exerted on this field of study in general. Since Dr. Hammett played a very active part not only in the establishment of our journal, but also in founding the Society for the Study of Development and Growth, it seems fitting that these acknowledgments should appear in the pages of GROWTH.

Fortunately, Dr. Hammett's retirement from the staff of the Institute does not mean that he has ceased to work in his chosen field; in fact, his latest contribution is so extensive that he has submitted only an abstract, which appears in this issue of GROWTH, and has arranged to reproduce the detailed data elsewhere. Though in poor health for many years, he continues also to serve actively on our editorial board.

We trust that he will be able to continue these activities for many years to come, and that we shall have the benefit of his stimulating and fruitful influence in the ever-widening field of growth study.

IRENE COREY DILLER, PH.D.,
Editor-in-Chief



FREDERICK S. HAMMETT

From a portrait painted at Provincetown, Massachusetts*
in 1942
by his friend, the late
MR. RICHARD E. MILLER

**Photograph taken by Mr. Pat Hallett.*

This is not to be a biography of Frederick S. Hammett—such an attempt would easily fill the pages of a large book—for his activities have spread into many fields, into many controversial subjects, and in each of them he has left a very definite impression. Rather, it is to be a small tribute to the imprint which he has left upon the thinking, the organization, and the work of the Lankenau Hospital Research Institute, now joined with The Institute for Cancer Research, Inc.—the staffs of which work as one on their problems, though nominally separated for convenience, legally and financially.

It was right after World War I that Frederick Hammett was working at the Wistar Institute, when at the Pathological Society of Philadelphia he presented a paper on pH measurements. With vim and vigor, with clarity and force, he demonstrated with test tubes, indicators and reagents his methods for determining this all-important factor in the control of many phenomena of living things. At that time I was pathologist to the Lankenau Hospital, but even before I had begun my internship I had already learned of the ravages of cancer, for my father, who was a general practitioner in Philadelphia for fifty-six years, had taken a vital interest in this problem. He had, himself, spent several years in Pathology at the Philadelphia General Hospital, and when I accepted an appointment as intern at the Lankenau Hospital, then the German Hospital of Philadelphia, he urged me to go back into the laboratory either in Pathology or Biochemistry, as a basis for an attack on cancer, which stood out then, as it does today, as one of the most challenging of medical problems. This was before the days of insulin or vitamins, or most of the hormones, sulfa drugs, penicillin and a host of others which have completely changed the practice of medicine.

After hearing Dr. Hammett present his paper, I wanted to clear up several technical points in connection with his work, and so I visited him at the Wistar Institute, but after having gone over these technical matters, our talk naturally gravitated toward cancer, the problem which was of such absorbing interest to me. "Why," said Hammett, "don't you try to gather together a few people, try to interest someone in building a laboratory at Lankenau Hospital, and start work on problems of growth and development." "Growth and development—both normal and abnormal?" I asked, and he nodded his head vigorously. Thus was crystallized the idea of the Lankenau Hospital Research Institute.

Fortunately, Mr. Rodman Wanamaker of Philadelphia was responsive to these ideas and generously donated funds for a laboratory building, which was dedicated in 1925 by Dr. John B. Deaver. In place of a bottle of champagne, he launched the laboratory by breaking a test tube of Fehling's solution on one of the albarene table tops of the laboratory. Meanwhile, Dr. Hammett had laid plans for what we would do if we ever got together. "You don't want all pathologists on your staff, just enough to carry on the clinical laboratory work properly, and with a bit of extra time for research in the pathological aspects of cancer. What you need is a biologist or two, an organic chemist or two, with assistants, with an animal colony, and with a fixed idea that the proper approach to the study of cancer is through growth and development."

Dr. Hammett came to the Lankenau Hospital Research Institute in September, 1927, as Scientific Director. He began his work with peas, beans, corn and onions. He asked the question: What are the chemical factors concerned in the control of growth and development? A question which the staff is still investigating. His immediate question within the frame of the larger one was: What are the chemical factors in cell division? "Naturally," said he, "I can use any living thing for this study, because all living things—at least most of them—have cells which divide by mitosis."

A good place to do fundamental research is in connection with a well-organized, active hospital, where the clinicians are constantly confronted with problems that they pass on to their confreres in the laboratory. All the members of the hospital, the Trustees, the staff, nurses and others, were much interested and pleased that cancer research—at that time an illusive, esoteric sort of subject—was to be investigated within the hospital. They expected, of course, that we would have cancers, human and perhaps some from lower animals, on our laboratory tables. Imagine their surprise when they visited the laboratory and found peas, beans, corn and onions growing in beakers containing botanical culture fluid. Both of us can still hear their snorts of derision and even remember many of the wise cracks when we met them at lunch or in other places around the hospital, but Fred Hammett insisted this was the correct way. He presented his findings, now a matter of record, at various biological societies where they were received as would be any other communication. When, however, he presented them at cancer research meetings, and

when jointly he and I presented them at meetings of pathologists, again we were met with raised eyebrows, wise cracks and the general statement, "If you want to do cancer research, why in the world don't you work with cancers?"

Interested persons may find it illuminating to scan the programs of the American Association for Cancer Research of 1932, 1933, 1934 and so on. A gradual change in the type of presentation will be noted. Now these concepts are part, not only of the programs of the meetings, but of the programs of research of all the laboratories in this country which profess to be doing cancer study.

In a year or more the work with peas, beans and corn, with paramecium, chick embryos and several other organisms thrown in for good measure, led to the development of an idea which helped wound-healing. Without telling the surgeons of the hospital in advance, Dr. Hammett and I tried this out on a patient with a leg ulcer and a fractured femur. When the fortunate results of the scheme were observed by our surgical colleagues, wise cracks were no longer in evidence. In fact, had Hammett wanted to work with elephants or anything else, they would have nodded understandingly and said, "Well, he is trying to understand what makes things grow."

What cells do under all sorts of circumstances was always one of his questions. In 1932 he began working at a small laboratory built like a Cape Cod cottage, in North Truro, on Cape Cod. With the help of the Women's Auxiliary, assistants were supplied who worked there from May 1st to September 30th each year. For sixteen years he guided the problems of the Marine Experimental Station of the Lankenau Hospital Research Institute and worked on them with the assistance of his students. The contributions of that laboratory to the growth problem are published in an extensive series of papers, many of which appeared in GROWTH.

Given to speculation, but usually constructively so, and given to quick generalizations, he was often in controversies, but many lines of endeavor and many modes of thought can be traced back directly to his work, his insistence and his persistence.

The present staff of our Institutes numbers 90 and is divided as Hammett said it should be many years ago. We approach the cancer problem through the conviction that it is part of the larger problem of growth and development. We believe that Frederick Hammett set the pace, and following his habit of wide speculation and quick

generalization, we find, as do many others, the foundations which he laid for us to be good for the investigation of the cancer problem as we see it today.

STANLEY P. REIMANN

The Origins of the Journal GROWTH

I think that I first met Fred Hammett in the summer of 1936. He had written to a number of people the country over, urging the desirability of a new journal to deal with the problems of "growth". It was his idea that it should cut through all the old categories based on either taxonomic compartments: Zoölogy, Botany, Entomology, or methods of approach: Chemistry, Psychology, Statistics, and should treat the one phenomenon, *growth* as exemplified in all fields and as approached by whatsoever method. I was one of those to whom he had written for opinion. The idea seemed sound in principle, but I seriously doubted the need for a new Journal. As a botanist I had found no difficulty in placing my papers in already established journals. The coverage and distribution seemed to be reasonably satisfactory. I had no personal complaint as things stood. I was far from convinced. But I had some slight acquaintance with Dr. Hammett's early work. I admired the way in which he had deduced the concept of sulf-hydryl control of growth phenomena from a careful study of a particular phenomenon—the toxicity of heavy metals for root-tips of onion—and had extended that concept to more general zoölogical and medical applications. I had already caught some glints of the flair for broad strokes which were so much a part of Fred's peculiar pattern of thought. And so, while expressing considerable doubt of the project, I consented to stop off at Cape Cod on my way from my summer in Vermont to look into the matter further. My wife and I made the trip by train and bus and found ourselves ensconced in a little backstreet boarding house in Provincetown.

I spent the better part of three days in the little North Truro Laboratory just reading,—letter after letter after letter. When I was through I said to him: "Dr. Hammett, these letters certainly show the existence of a real need which I had not in the least suspected. Obviously many workers, particularly medical men interested in general growth phenomena, mathematicians interested in growth, biologists interested in certain types of statistical studies and

similar intermediate fields are finding their papers out of place in all the present journals. There *is* a need for a new and different journal. It is still true that this need has been less urgently felt by botanists than in some other fields, and it is rather doubtful if many botanists will make use of such a journal. But the need *is* there, much to my surprise. If such a journal is established I will do what I can to help you make a success of it."

Dr. Hammett respected my doubts and my insistence on real evidence. Those three days and the two or three that followed them established a firm friendship and a mutual respect which, although it has suffered some occasional and superficial set-backs, has nevertheless, underneath, endured. During that time he drove me to Woods Hole where we met with a number of others who were interested in the project and discussed the possibility of founding such a journal, who should be its editors, what should be its policies. Afterwards he went to the hospital for a pneumothorax and I returned to Provincetown.

I do not remember all of those who met at Woods Hole at that time. Edward Adolph, Charles Davenport, Fred and myself are the only ones I remember. There was a good deal of opposition, some doubts even more serious than my own, but we went away with the feeling that it was indeed a project which justified support, that Fred Hammett had rightly read the pulse of an inchoate but nevertheless significant minority of biological thought and that he should be encouraged to go ahead with preliminary plans.

Fred did so during the next year. Under his leadership and largely by correspondence we set up a provisional board of editors, made a survey of publication problems, canvassed the question of preliminary finance and were ready to begin the final preparations for publication. Fred Hammett, as originator of the plan and its mainspring, was to be Editor-in-Chief, but both to reduce his work (for he was already a very sick man) and also to avoid as much as possible the impression that the Journal might be no more than a "house organ" of the Lankenau Hospital Research Institute, it was decided that John Berrill should be managing editor and that the journal should be issued from Montreal, rather than from either Philadelphia or Provincetown. Dr. Lawrence Frank and the Macy Foundation were our "Angels".

We published our first number in April, 1937. Fred Hammett wrote the "christening address", as indeed he should. It is worth rereading. The broad principles, the objectives, the general plan were clearly and cogently stated. He knew what he wanted to say and how to say it. We, his co-workers knew that it was worth saying. That address was not seriously marred by Fred's later tendency to Provincetown jargon. We were off.

We met again in 1937 in Provincetown, at the Colonial Inn. I have the roster of that meeting: John Berrill, Leigh Hoadley, Richard Scammon, Lawrence Frank, Charles Davenport, Leo Loeb, Stuart Courtis, Sam Brody, Otto Rahn, Fred Hammett and myself. There was none of the uncertainty and doubt that had been in the background at the earlier meeting. We knew that we were on the right track. Our problems were of means and details. We discussed the possibility of a society but set that aside for the moment.

GROWTH is first, last and always Fred Hammett's creation. The idea was his. The spade work in putting that idea across to others was his. The objectives and the plan of the journal were his. He led it through its first year as editor. The plan which he laid out remains today unchanged. Let us not forget that the good which there is in that project we owe to the vision, the effort, the pertinacity and the self-sacrifice of one man, of infirm body and sometimes eccentric mind, but of indomitable spirit.

PHILIP R. WHITE

FOUNDING OF THE SOCIETY FOR STUDY OF DEVELOPMENT AND GROWTH

No review of the activities of Dr. F. S. Hammett would be complete without acknowledgment of the important part he played in the formation of the "Society for the Study of Development and Growth". This Society had its origin in a symposium held in North Truro, Massachusetts, from August 7 to August 11, 1939. Over the past 11 years it has sponsored a most stimulating series of symposia with topics which have appealed to a group of biologists and others, interested in many diverse fields. Those of us who attended that first meeting, sponsored by the Editors of GROWTH, still recall the enthusiasm of those present. All expressed their gratitude to Dr. Hammett for his tireless efforts for their comfort, for their entertainment and for their enjoyment of the sessions. At the close of the last session the assembly appointed a committee which, under the chairmanship of Dr. Warren H. Lewis, should concern itself with the details of organization of the Society and with the arrangements for a second symposium to be held the following year. That meeting was held at Salisbury Cove, Maine, from June 20 to June 26, 1940.

It is quite proper that as the years pass and as the responsibility for projects passes from one group to another the names of those responsible for the initiation of the project should be forgotten. They have but sown the seed and nursed the seedling. That was the rôle of Dr. Hammett. The virility of the seed and the fertility of the ground is attested by the sturdy growth of the plant.

LEIGH HOADLEY

Growth, 1950, **14**, 263-264.

QUANTITATIVE GROWTH IN OBELIA COLONIES IN CULTURE: I. RATES OF BASIC ACTIVITIES

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Earlier report describes the *structural changes* incident to the growth and life-cycle progression of Obelia hydranths and gonangia. It shows how each definitive change is a mark of dominance of a separate basic growth or life-cycle activity (1).

This report deals with the *rates of expression* of the activities, as derived from 457,916 colonial-living hydranths and 35,093 gonangia in 24-hour time-slices in culture; over the months from April-May to September for 7 years, by periods (4 per month), months, and years.

The basic finding is that the rate of each is individual and distinct from each other. Thus—the 24-hour expression of potentiality of each in overall (all manifestations in all periods, months, and years) in the hydranths is: maintenance, 96.7%; differentiation, 88.3%; organization, 83.7%; catabolism, 82.1%; proliferation, 60.2%; initiation, 27.3%; regression, 24.7%. In the gonangia it is: proliferation, 58.5%; differentiation, 33.4%; organization, 25.9%; initiation, 6.3%. The differences are real, because consistently present in the periods, months and years separately, regardless of variations. And because rates are those of activities occurring simultaneously in the same time-slices and environments.

Thus the differences as such are endogenously determined. The products of innately different sets of chemical processes. And the data are *quantitative* substantiation of the principle postulated from *qualitative* record (1) that growth is not single but multiple: the summated expression of separate but integrated activities (2:3). Which has not hitherto been had in totality in one and the same organism.

It is axiomatic that this applies to growth wherever expressed, and should always be applied to analysis of the property. For, *inter*

alia, the significance of any condition or compound to growth rests on which of the activities is primarily reactive thereto.

Adequate evidence is needed. Therefore such is given *in toto*. In 16 pp. of Tables; 2 of Charts; 7 of Plates; 28 of summarizing Text; 74 of explanatory Appendices; and 7 of References. The Appendices give and discuss: A. Source of Data; B. Species Purity; C. Care of Colonies; D. Elements of the Record; E. Getting the Record; F. The Raw Data; G. Culture Effect; H. Absolute Rates; and I. Relative Rates.

A report of this nature and length is too much for any Journal to publish. So the MS has been deposited with the American Documentation Institute, 1719 N Street, N.W., Washington 6, D. C. From which copy may be obtained by ordering Document No. 3142 remitting $3.96 for microfilm (images one inch high on standard 35 mm. motion picture film readable in microfilm reader or under low power microscope), or $39.60 for photocopies (6 x 8 inches readable without optical aid).

References

1. Hammett, F. S. 1943. Description of Obelia and its Growth. *Growth,* **7**, 331-399.
2. ———. 1942. The Growth of an Obelia Hydranth. Introduction. *Growth,* **6**, 59-123.
3. ———. 1936. The Nature of Growth. Science Press, Lancaster, Pa.

Growth, 1950, **14**, 265-275.

A STUDY IN TRANSPLANTED RAT AND SPONTANEOUS DOG TUMORS OF GROWTH ACCELERATING AND INHIBITING FACTORS FROM DUOJEJUNAL TISSUE OF HOGS, SHEEP AND DOGS

PART II

ROBERT L. LADD, M.D., MARK T. GOLDSTINE, M.D. AND OCIE R. BURNS

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The results obtained in the preliminary experiments which were reported in Part I of this study (8) do not offer any information or suggestions about the sequence of events which precede neoplastic transformation in cells, but do suggest that certain naturally occurring substances may modify the growth rate of tumorous tissues.

This probability is enhanced by the work of Roffo (11), Boyland (3), Brues *et al.* (4), Beard (1) and a number of other investigators.

In this report we present the results obtained by treating tumor bearing rats with several fractions from duojejunal extracts.

In some of the animal experiments which are to be described, the number of tumor bearing rats available at the time was not sufficient for a large experiment, but we believe the resulting data are not without significance.

In the first experiments our measurements were made by calipering the maximum diameter of the tumor. For obvious reason this method of measuring tumor growth is not wholly satisfactory For this series of experiments we made a measuring gauge with an adjusting loop terminal the stem of which was graduated in millimeters. By the use of this instrument we were able to measure quite accurately the maximum circumference of the tumor. After measuring the circumference of the normal hind leg we subtract that measurement from the circumference of the tumor leg. Thus we obtain a measurement of increase in circumference due to absolute tumor growth.

By measuring the tumor at the start and periodically throughout the experiment, the growth rate of the tumor was obtained. The sum of the individual increases or decreases in tumor size for the period between successive measuring dates, divided by the number of rats and the number of days involved the ratio:

$$\frac{\text{mm. increase in tumor size}}{\text{rats} \times \text{days}}$$

This we call our tumor growth index or TGI. The overall TGI was calculated for each experiment by multiplying the individual TGI's by their respective number of days, summing these products, and dividing the sum by the total days duration of the experiment. As will appear in the data to follow it is possible to have a negative TGI, e.g., when a predominate number of tumors is regressing during any given period. By comparing TGI's computed for treated animals with those obtained for the untreated, for any given period, we feel that we secure a reliable index of tumor growth acceleration or inhibition.

The data presented in the first paper show that the percentage of positive transplants was about 76.0%. In a series of recent experiments in which 201 rats were inoculated with Jense Sarcoma tumor pulp, 196 tumors developed or 97.5%.

Within a two-year period we had 3 spontaneous regressions in a total of 392 animals used as controls. This small percentage of spontaneous regressions is, we believe, due to the fact that this tumor has passed through this particular strain of animals for a ten-year period.

In Part I two methods of separating the accelerating and inhibiting factors present in duojejunal extracts were discussed. These were dialysis and adsorption, both of which depend upon the size of the molecular aggregates present.

In addition to the use of aluminum cream, three other adsorbents, permutit, Lloyd's reagent, and activated bentonite were used in our fractionation experiments.

The extracts used in the first experiment were prepared in the following manner. The minced tissue, plus a small amount of water, was heated on the steam bath for one hour. The liquid and solid portions were then separated and the liquid discarded. The solid portion was digested with pepsin in the usual manner, and then

with trypsin. Alcohol was added to a 10% concentration during the digestion with trypsin. The digest was filtered clear and concentrated to a paste. A 2% aqueous solution of this paste was prepared and divided into two parts, PD-1 and PD-2. PD-1, preserved with merthiolate, was used as control therapy in the fractionation experiment. The pH of the solution was 7.6 and the nitrogen content 215.6 mg. per 100 ml.

The second portion was passed through a column of permutit. The permutit had previously been washed with water, 2% acetic acid, and again with water. The filtrate was collected, preserved with merthiolate, and labeled PD-2. The pH of this solution plus merthiolate was 8.2 and the nitrogen content 63 mg. per 100 ml.

After PD-2 was collected the column was washed with a saturated KCl solution. This release was labeled PD-3. The pH of this solution plus merthiolate was 7.9 and the nitrogen content was 54 mg. per 100 ml. The results of this experiment are shown in Table 1.

TABLE 1
THE EFFECTS PRODUCED BY FHD FRACTIONS ON TUMOR GROWTH IN RATS

No. of Rats	Therapy	Days of Expt.	Overall TGI
10	PD-1	33	2.15
10	PD-2	33	1.94
10	PD-3	33	2.20
10	Controls	33	2.49

From data shown in Table 1, it is apparent that the administration of solution PD-1, without modification, caused some inhibition of tumor growth. The removal of nitrogenous bases with the aid of permutit increased the inhibitory properties of the original solution. The release, which contained the separated nitrogenous bases, had less inhibitory activity than the control therapy.

This method for separating amines from other nitrogeneous substances was developed by Whitehorn (10).

The solutions used in the second experiment were made in the following manner. A pepsin, -trypsin digest, prepared as described in experiment No. 1, was further digested with lipase. Instead of concentrating the filtered digest to a paste, the filtrate was raised to an alcohol concentration of 70%. Both the precipitate which formed and the remaining filtrate were used in subsequent experiments. The precipitate was suspended in a small volume of water

with the aid of a Waring blendor and the suspension dried on glass plates to a pale yellow, scaly product which was easily powdered. The filtrate, containing the alcohol-soluble substances was dried, labeled TD and reserved for subsequent experiments.

A 1% aqueous solution of the powdered alcohol-insoluble material was prepared and labeled 1-70. Lloyd's reagent, after treatment with 1.7 N HCl was placed in the adsorption column and to this was added a part of the solution of 1-70. The remaining portion was used as control therapy.

The material adsorbed by Lloyd's reagent was released with 1% pyridine and dried. A 1% aqueous solution of the dried material plus merthiolate was used in the animal experiment. This solution was labeled 1-70-R. The results of this experiment are shown in Table 2.

TABLE 2
EFFECTS OF FHD FRACTIONS ON TUMOR GROWTH IN RATS

No. of Rats	Therapy	Days of Expt.	Overall TGI
10	1-70	21	2.76
10	Controls	21	2.09
6	1-70-R*	20	1.56
7	Controls	20	2.45

*Recovery.

From the data shown in Table 2 it appears that a solution with tumor growth accelerating properties may be so modified as to show growth inhibition. Bergdoll and Doty (2) used Lloyd's reagent for the adsorption of basic amino acids and sodium bicarbonate as the eluant.

In the third experiment of this series, the adsorbent employed was activated bentonite.

The solutions used were prepared from PHD in the following manner. After defatting with acetone the material was extracted with sulfuric acid. The extract was filtered clear and shaken with a suitable quantity of activated bentonite. The bentonite was removed by filtration and the filtrate was dried on glass plates. A 1% aqueous solution of the dry powder was prepared, preserved with merthiolate, and labeled PHD.F-1.

The bentonite residue was extracted with 5% pyridine, centrifuged, and the solid residue discarded. The pyridine was removed from the supernatant liquid by evaporation and this was followed by dialysis.

When the dialysed solution was titrated to pH 4.4 with HCl, an amorphous precipitate formed. This precipitate was separated by centrifugation and suspended in twice its weight of water. The pH of the suspension was adjusted to 5.0 with N NaOH and a part of the precipitate redissolved. The solution was filtered and the filtrate dried at 50°C. A 1% aqueous solution of the dried material was prepared, preserved with merthiolate and labeled PHD.F-2. The results of this experiment are shown in Table 3.

TABLE 3
EFFECTS OF PHD FRACTIONS ON TUMOR GROWTH IN RATS

No. of Rats	Therapy	Days of Expt.	Overall TGI
3	PHD.F-1	11	1.72
3	PHD.F-2	11	—0.636
3	Controls	11	0.236

From the data presented in Table 3, it is apparent that the unadsorbed material, PHD.F-1, was accelerating while that adsorbed on activated bentonite and released, PHD.F-2, was inhibiting to such an extent that a negative TGI resulted.

Kunitz (7) employed activate bentonite as the adsorbent and pyridine as the eluant in extracting a protease inhibitor from soy beans.

Observations, made during a number of animal experiments, have indicated that after drying the fresh tissue or heating the tissue extract, preparations were obtained which were much more potent as tumor growth inhibitors than unheated extracts prepared from fresh tissue. Due to the marked tendency of this tissue toward bacterial decomposition, the drying process was often carried on at such a temperature that denaturation of native protein might be expected.

With denaturation, the unfolding of the protein molecule would perhaps result in the exposure of certain molecular groups, particularly the sulfhydryl groups, and thus increase the reactivity of such radicals. In the following experiment the effect of heat on extracts of fresh tissues is demonstrated.

Fresh tissue was extracted with ice cold water and stored overnight in the refrigerator. A second extraction was made in a similar manner. The combined filtrates from the extractions had a pH of 5.91. This solution was heated to 70°C., for 30 minutes. Coagulation was observed at 55°C. The precipitate was removed by filtration, the pH

of the filtrate was adjusted to 6.3 and alcohol was added to 67% concentration.

The precipitate which formed was collected, suspended in distilled water and dialysed for three days against distilled water. The bag content was filtered and dried. A 1% aqueous solution was prepared from the concentrate and labeled FHD-1.

FHD-1 was divided into two portions, one of which (FHD-1) was used as control therapy and the second portion was autoclaved at 19 lbs. pressure for 22 minutes. This fraction was labeled FHD-2. The results of this experiment are shown in Table 4.

TABLE 4
THE EFFECT OF HEAT ON AN FHD EXTRACT

No. of Rats	Therapy	Days of Expt.	Overall TGI
6	FHD-1	28	2.35
6	FHD-2	28	1.55
9	Controls	28	1.93

As may be noted in Table 4, a water extract of fresh tissue, heated to 70°C. for 30 minutes, is definitely growth stimulatory. After autoclaving, this extract shows growth inhibition. From this experiment it may be inferred that the growth stimulating factor is thermolabile and the inhibitory factor thermostable.

In an earlier experiment the pseudoglobulin fraction from a water extract was tested in animals for growth regulatory factors. The results obtained from this experiment indicated that such a solution was growth inhibiting. Loss of data made it necessary that the experiment be repeated and it was deemed desirable to include in this series of experiments, a study of additional fractions obtained from ammonium sulfate precipitation.

Freund and Kaminer (5) made the interesting observation that normal serum contained a carcinolytic factor and that on fractionation of the serum, this substance was to be found in the euglobulin fraction. Normal organs have also yielded a carcinolytic factor.

The ammonium sulfate fractions used in this experiment were prepared in the usual manner from an aqueous extract. The euglobulin fraction was labeled 0-33, the pseudoglobulin fraction, 33-50, and a third fraction was prepared and labeled 33-100. The results of this experiment are shown in Table 5.

TABLE 5
EFFECTS OF FRACTIONAL AMMONIUM SULFATE PRECIPITATION OF FHD ON TUMOR GROWTH IN RATS

No. of Rats	Therapy	Days of Expt.	Overall TGI
5	0-33	38	2.10
3	Controls	38	1.79
6	33-50*	42	0.69
6	33-100**	42	0.89
7	Controls	42	1.66

*Recovery.
**Recoveries.

The data shown in Table 5 indicate that the 0-33 fraction is growth stimulating, the 33-50 retarding and the 33-100 fraction also retarding.

In the five fractionation experiments reported above, evidence is presented from which some physico-chemical properties of the tumor growth regulatory factors may be adduced. The potency of a preparation as a tumor growth inhibitor is enhanced by the removal of nitrogenous bases. The gross separation of the molecular aggregates and the elimination of those of smaller dimensions also increases the potency of the solution. The pseudoglobulin fraction was more effective than the euglobulin fraction and heating to a temperature above that of the steam bath apparently destroyed the accelerator and left the activity of the inhibitor unimpaired.

Hammett (6) and Reiman & Hammett (9) have reported certain results obtained from extensive studies of the mechanism and chemistry of normal growth. It is obvious that the lack of knowledge of the physiology and biochemistry of normal growth impedes the study of malignant growth.

This group of workers found that organic compounds containing sulfur could be treated in such a manner as to accelerate or retard growth by increase in cell numbers. They also found that the degree of oxidation of the sulfur was the controlling factor. More specifically, they pointed out that the -SH and the H-SS-H groups were accelerating and that sulfones were inert, whereas sulfoxides and more highly oxidized organic sulfur radicals were inhibiting. However, when sulfur was oxidized to the sulfate level, the products were again inert.

If the normal growth mechanism is intimately connected with the reversible oxidation of organic compounds, it is not illogical to postulate that malignant growth may be a chemical deviation from

the normal process, characterized by an impairment of one or more of the several systems important for biological oxidations.

In the preparations used in Expt. No. 2, the alcohol insoluble fraction of a total digest was used and a filtrate containing alcohol soluble substances was labeled TD and reserved for a subsequent experiment. A 1% aqueous solution of TD was prepared ond labeled TD-1. Analysis of this solution disclosed the presence of disulfide groups. The results of the animal experiment with TD-1 are shown in Table 6.

TABLE 6
ANIMAL EXPT. NO. 6

No. of Rats	Therapy	Days of Expt.	Overall TGI
10	TD-1	16	2.34
10	Controls	16	2.09

The stimulation of tumor growth in rats by a solution containing disulfide groups is demonstrated.

The partially oxidized preparations used in the remaining part of Expt. No. 6, were made by a modification of the method used by Robertson *et al.* (13).

TD, in oleic acid solution, was oxidized by aeration for a 5-day period and the resulting product was filtered and labeled TD-2. A portion of TD-2 was extracted with an equal volume of benzol and the mixture filtered through paper. Benzol was removed from the filtrate and the product labeled TD-3. The benzol insoluble fraction was made into a 2% solution in corn oil and labeled TD-4.

The results of the animal experiment with TD-T are shown in Table 7.

TABLE 7
EFFECTS PRODUCED BY A PARTIALLY OXIDIZED FHD FRACTION ON TUMOR GROWTH IN RATS
Animal Experiment No. 6

No. of Rats	Therapy	Days of Expt.	Overall TGI
10	TD-2	32	1.98
10	Controls	32	2.20

Oxidation of TD has, apparently, changed a growth promoting preparation to one showing a slight measure of growth inhibition.

The results obtained in the animal experiment with TD-3, the benzene soluble fraction, are shown in Table 8.

In animals treated with the benzene soluble fraction, the tumor grew more rapidly than did those in the controls.

TABLE 8
ANIMAL EXPT. NO. 6

No. of Rats	Therapy	Days of Expt.	Overall TGI
4	TD-3	18	2.21
4	Controls	18	1.96

The results of the animal experiment with TD-4 are shown in Table 9.

TABLE 9
EFFECTS PRODUCED BY FHD FRACTION ON TUMOR GROWTH IN RATS

No. of Rats	Therapy	Days of Expt.	Overall TGI
4	TD-4	47	1.08
6	Controls	47	1.40

With the benzene insoluble fraction, transferred to corn oil, tumor growth inhibition is effected.

The results obtained by the partial oxidation of TD, indicate that the growth inhibitory property of the extract may be associated with the degree of oxidation of sulfur containing compounds. For this reason an additional preparation was included in this series. Toennies (12) studied the possibility of oxidizing cysteine to the sulfenic acid level by rapid oxidation in non-aqueous media. He was able to identify the chief oxidation product as a sulfur derivative at the sulfenic acid level. According to Hammett, at this level of oxidation the preparation should be growth inhibiting.

The oxidation of TD to a higher level than that obtained in TD-2, was attempted by the use of hydrogen peroxide. The resulting product was transferred to oil and labeled TD-5. In Table 10, Expt. No. 6, the results obtained with TD-5 are given.

TABLE 10
ANIMAL EXPT. NO. 6

No. of Rats	Therapy	Days of Expt.	Overall TGI
6	TD-5	27	2.21
6	Controls	27	2.75

The TGI differential as computed from Toble 9 is 0.32 and that from Table 10 is 0.54. Apparently the most effective level of oxidation had not been reached in TD-4 and this may also be true in TD-5.

In Expt. No. 6, 34 rats were treated and 36 were used as controls. It is interesting to note that the TGI differential ranged in numerical

value from —0.25 to +0.54 where the positive sign indicates inhibition and the negative, stimulation.

Summary

In the experiments included in this report a total of 200 tumor bearing rats were used, 115 were treated, and 85 were used as controls. The average TGI value for the controls was 1.92, that for the 33 animals showing faster growth rate, 2.28, and that for the remaining 82 rats showing a slower growth rate was 1.47.

From this overall analysis of the data presented in the ten tables included in this report, the experimental evidence is that this tissue contains substances which will modify the growth rate of tumors in experimental animals.

In Expt. No. 6, by the partial oxidation of sulfur containing radicals, growth inhibition is enhanced and it appears that the degree of inhibition obtained is closely related to the oxidation level of the organic sulfur groups contained in the extracts.

It is obvious that results obtained with transplanted tumors may differ from those obtained with spontaneous tumors and only with great caution can the results of animal experiments be translated to human pathology.

Further experiments are now in progress and C3H mice from The Jackson Memorial Hospital are being used as the experimental animals.

References

1. Beard, H. H. Effect of injection of individual amino acids upon Emge sarcoma in rats. Federation Proc. 2:58-1943a.
2. Bergdoll, M. S., & Doty, D. M. "Chromatography in the Separation and Determination of the Basic Amino Acids." Ind. Eng.
3. Boyland, E. 1941. Experiments on chemotherapy of cancer: effect of muscle extract and aliphatic bases. *Biochem. Jour.* **35**, 1283-88.
4. Brues, A. M., Subbarow, Y., Jackson, E. B., & Aub, J. C. 1940. *Jour. Expt. Med.* **71**, 423-438.
5. Freund, E., & Kaminer, G. 1925. "Die biochemische Grundlagen der Karzinomdisposition." Springer, Vienna.
6. Hammett, F. S. 1930. "The Natural Chemical Equilibrium Regulative of Growth by Increase in Cell Numbers." *Protoplasma,* **11**, 382.
7. Kunitz, M. 1945. "Crystallization of a Trypsin Inhibitor from Soy-bean." *Science,* **101**, 668.
8. Ladd-Goldstine-Burns. 1950. *Growth,* **14**, 155-166.
9. Reiman, S. P., & Hammett, F. S. 1940. "Chemical Specificity in Growth and Development." *Ann. Int. Med.,* **13**, 1483.

10. ROBERTSON, W. B., HARTWELL, J. L., & KORNBERG, S. 1944. "The Oxidation of Thioethers by Unsaturated Fatty Acids." *J. Am. Chem. Soc.*, **66**, 1894.

11. ROFFO, A. H. 1937. Regression et elimination des tumeurs malignes du rat por L'hydrolise de muscle strie. *Assoc. Franc pour L'etude du cancer bul.* **26**, 602-604.

11*a*. ———. 1938. Action of hydrolysate of striped muscle on malignant tumors. *Lancet*, **2**, 184-187.

12. TOENNIES, G. "Oxidation of Cysteine in Non-aqueous Media."

13. WHITEHORN, J. C. 1923. "'Permutit' as a Reagent for Amines." *Biol. Chem.* **56**, 751.

Growth, 1950, **14**, 277-294.

CYTOPLASMIC GROWTH IN THE MUSCLE FIBRES OF LARVAE OF *DROSOPHILA MELANOGASTER*

Abbreviated title

CYTOPLASMIC GROWTH

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INTRODUCTION

When a cell has undergone mitotic division each of the resulting cells receives an equal amount of cytoplasm, in most cases exactly one-half of that of the original mother cell. The decrease in amount of cytoplasm is made up in the newly established daughter cells as soon as possible by a process commonly referred to as cell growth. This phase of cell activity has received up to now very little attention, since most of the interest of the investigators was directed toward the involved mechanism of cell division. This exclusive interest in the nuclei is chiefly due to the fact that in most cells the cytoplasm is optically empty, with the exception of cytoplasmic inclusions in some types of cells. Consequently, few clues are available as to the mechanism of cytoplasmic growth.

Conditions are somewhat better in the muscle cells because of the exceptional differentiation of their cytoplasm. The cross striation and the myofibrils can be distinguished with the regular microscope; the submicroscopic structure can be investigated with the polarization and electron microscope; and chemical differences are accessible to cytochemical tests. Under these conditions it should be possible to study the growth of muscle cells, with the hope of learning something about the process of cytoplasmic growth in general.

Muscles of insects are favorable material for this investigation, since their larvae, especially those of the holometabolous type, grow very rapidly, which makes it easy to follow the process in a short time. Every one of their muscles is equivalent to a giant cell with ten and more nuclei, and analogous to single muscle fibres of ver-

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tebrate muscles. They are attached to the larval skin where they form a complex system, which is already present when the individual hatches from the egg. This makes it possible to select a few muscles, which are particularly well suited for the study under certain points of view, and to follow their growth during successive instars. Since no nuclear division takes place during the larval period, we have a case of cytoplasmic growth under almost ideal conditions.

As material, larvae of *Drosophila melanogaster* were chosen, because, as already pointed out by Wilson (1939) this material is admirably suited for investigations of all phases of growth. Despite the apparent small size of the entire organism the cells are large enough to permit thorough microscopic study, which is particularly true for the muscle cells. Furthermore, there is a careful statistical investigation of the growth of the whole larva, carried out by Alpatov (1929), on which the present study of the growth of muscle cells could be based.

Very little work has been done on the problem as stated. There is a voluminous literature on the histogenesis of muscle tissue tracing the development of muscle cells from the undifferentiated myoblasts to the mature cells, the summary of which can be found in any larger treatise on histology, but very little is known about the growth of already differentiated cells. For, it should be borne in mind that there is a difference between the differentiation, which is only one of the fundamental processes of what is commonly referred to as "growth", and mere increase in size which has been termed by Hammett (1942) "Substance Increment" or "Anabolic Growth". It can be assumed that this sort of process or series of processes are the same wherever they are found. It occurs always after cell division when the two daughter cells acquire the original size of the mother cell. It occurs also in what we may call in a more popular way "growth of muscle cells in Drosophila larvae". When these larvae hatch from the egg they start crawling about, which indicates that their muscles are already differentiated. During the ensuing instars the muscles increase in size only and the amount of living substance is greatly enlarged.

Several workers have studied the growth of isolated muscle fibres in tissue cultures. W. H. Lewis (1939) gave a summary of the results obtained. This author observed a steady, although slow, increase in length of the muscle explant without any apparent diminution of its thickness during periods of weeks and months. There was a

great increase in the protoplasmic mass without any cell division. Most of the fibres showed the well known phenomenon of dedifferentiation of tissue culture cells by losing their cross striations. There is no mention of the mechanism of growth involved.

An early worker who comes close to the problem is Weismann (1864). He devoted about one page of his paper on postembryologic development of the Muscidae to the question of how the musculature of the larvae of the flesh fly *Musca vomatoria* increases in size. He noticed that the larvae when emerging from the eggs are endowed with functional muscles, the nuclei of which do not increase in number, but in size only during the ensuing period of larval growth. Weismann noticed also the extremely large nucleoli in these nuclei. Myofibrils and their elongation, are not mentioned at all. More recently Heidenhain (1919) investigated the growth of muscle cells in larvae of salamanders and the trout. He comes to the conclusion that elongation takes place at the ends of the fibres. No further reference to the problem of growth of differentiated muscle cells could be found in the literature of recent years.

Material and Methods

Larvae of *Drosophila melanogaster* from a wild stock culture were raised in Petri dishes on the usual culture medium. The dishes were placed under a plastic microscope jar which harboured the flies, and the animals allowed to deposit their eggs on the surface of the medium. The food supply was abundant and the moisture carefully balanced, which allowed to each larva food enough to attain its maximum length at a given stage of development. The temperature at which the larvae were raised was about 20° C. At intervals of several hours the larvae were collected from the dishes and killed by plunging them into the fixing medium which was a 10% solution of formol in water. This fixative preserves well the properties of striated muscle tissue for polarization optical studies. The musculature became uniformly relaxed by this way of killing, and the muscles were fixed in the relaxed condition.

Most of the investigations were carried out on whole mounts of larval skins. The fixed animals were placed upon a microscope slide and slit open lengthwise. The inner organs were removed and the empty larval skins washed in water to remove tissue debris and fat droplets. After this the skins were transferred to another slide coated

with albumin fixative. The slides were passed rapidly over a small flame in order to coagulate the albumin, whereby the material adhered to the slides, which then were run up to xylene and embedded in balsam. By this method every muscle was mounted in its full length and natural position since all of the fibres were still attached to the larval skins. In this way comparative studies and measurements could be made easily.

Many observations were made in polarized light. For this purpose the microscope was equipped with polaroid filters. For the study of myofibrils the material was stained with safranin in aqueous solution for several hours, and mounted in balsam as described above. It was found that material prepared in this way could be studied with advantage between crossed polaroids, opening the analyser just a bit to permit some natural light to enter the microscope. Specimens destined to the examination of nuclei were prepared by the method of Kurnick and Ris (1948). Acetic orcein was mixed with fast green in alcoholic solution in the proportion 2:1; some drops of saturated NaCl solution were added. The material was permitted to stain for two hours and then embedded in balsam. The nuclei stain dark brown, the nucleoli and the cytoplasm become green.

Observations and Results

1. *The Anatomy of the Muscular System*

Alpatov (1929) has shown that in Drosophila larvae there is a steady increase in length during each of the instars, and that length is gained also in an abrupt way during the process of moulting. In order to take into account this periodicity, specimens were selected for closer study at both the time of abrupt stretching and of steady growth. This was accomplished by taking the specimens shortly after moulting, in the middle of each instar, and shortly before the next moult. From the first instar only specimens from the end of this period were considered, since the younger ones were found to be too delicate to stand all the phases of the preparation process. In the third instar the upper limit was the imminent pupation.

In sorting larvae according to instars, the criteria given by Alpatov were followed. It was found that the length of the mouth armature is the easiest to use in routine work. At a magnification of 10x, the mouth parts of the first instar larvae were scarcely visible, while

those of larvae in the second stage could be well distinguished in their outlines. In the third instar they appear clearly visible also in details. In doubtful cases measurement with the ocular micrometer was resorted to.

There was a noticeable variation in the speed of growth in different individuals despite equal conditions in the environment. Some of the animals grew rapidly and rushed through the different stages in short periods. Others were extremely slow and needed about twice the time. It was found, however, that in both cases the final length attained in a given instar was nearly constant for a given lot of larvae, so that in all cases animals having reached a certain body length proceeded to moulting or to pupation. The variation of the body length depended only upon environmental conditions, such as moisture, abundance of food, temperature, and the like. For this reason it was convenient to compare not larvae of the same absolute age as expressed in hours, but animals of the same state of development as expressed by body length in mm. Table 1 summarizes the measurements of larvae raised and fixed under conditions specified above. There is no mention of the type and degree of variation, since it has been described fully by Alpatov.

The muscular system of Drosophila larvae consists of a complex texture of longitudinal, oblique, and transversal fibres. All of the muscles are attached to the larval skin. The arrangement of the muscles is metameric in the thorax and in the abdomen in each segment, with the exception of the eighth abdominal segment which shows a considerable modification. Figure 1 gives an over-all view of the whole muscle system. It is evident that not every one of the many hundreds of muscles could be examined in all detail, but a few of them were selected. The choice was guided by considering the particular feature for which they were to be examined. All of the se-

TABLE 1

Measurements of larvae at different stages in mm. Under length of mouth armature is understood the distance between the top of the large hook and the incision of the hypostomial sclerite.

Instar	Body length		Mouth Armature
	Beginning	End	
I	0.58	1.26	0.108
II	1.44	2.70	0.216
III	2.88	4.35	0.360

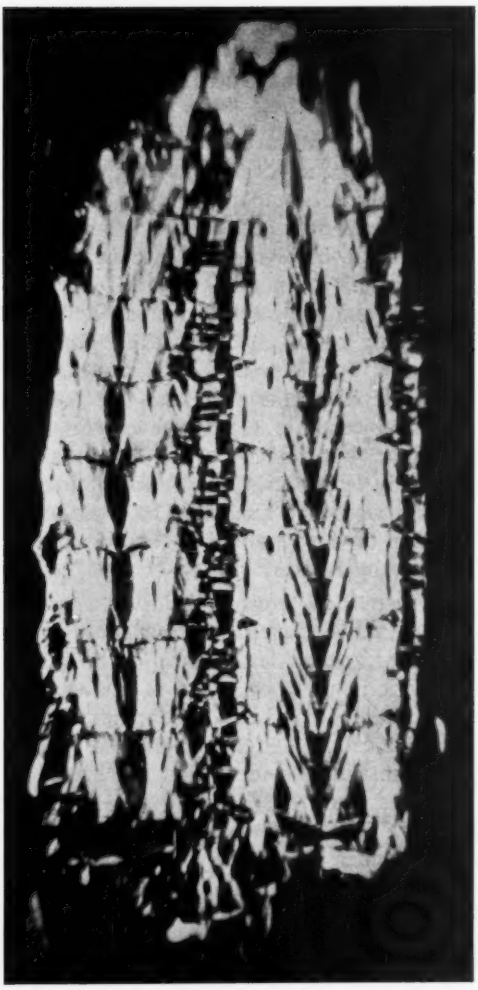

FIGURE 1

Over-all view of a third instar larval skin and muscular system of a larva of *Drosophila melanogaster* in polarized light. The dorsal portion is to the left, the lateral part in the middle, the ventral portion to the right. The other lateral portion is not shown. The eighth abdominal segment suffered from the process of mounting. The magnification is X 45.

lected fibres were taken from the abdominal segments. In order to exclude any variation which might exist between the muscles of different abdominal segments, only those of the sixth segment were studied. The terminology follows that employed by Hertweck (1931).

Cross striations were investigated in the Musculus dorsalis obliquus externus (abbreviated "dorsal muscle"). This consists of a double pair of single fibres situated "exteriorly", i.e., directly under the cuticle, running in a slightly oblique direction from the anterior to the posterior border of each segment. In the posterior-anterior direction the two pairs of fibres at each side of the dorsum are inclined toward the mid-dorsal line. Toward the body cavity the "externus" is covered by two other pairs of fibres, which are collectively termed the Musculus dorsalis obliquus internus. They are inclined in the opposite direction. To work out the growth of myofibrils ventral fibres were chosen, because they are very flat and less liable to obscure the picture by several layers of myofibrils. There are three pairs of fibres collectively termed Musculus obliquus ventralis (abbreviated "ventral muscle"). One of these pairs stands out in a particular fashion forming a sort of X-like structure in each segment. This pair of fibres has been selected for the examination of myofibrils. The nuclei, finally, were investigated in the Musculus longitudinalis ventro-lateralis (abbreviated "lateral muscle"). This is a group of three fibres running in a straight line in the anterior-posterior direction of each segment, neighboring on either side the oblique ventral muscle. The central fibre was chosen for the study of the nuclei. Figure 2 shows in diagrammatic fashion the relative location of the muscles in an abdominal segment.

2. *The Cross Striations*

The cross striations are beyond doubt the most striking feature of skeletal muscle fibres, so their development and behavior during growth was studied first. The length of the dorsal muscle was measured in larvae arranged in a series of increasing total body lengths, and in each of the muscles measured, the number of cross bands was counted.

There is no great difficulty in ascertaining the lengths of these muscle fibres. There is a certain degree of inaccuracy resulting from the nature of the measuring tool which did not permit a higher accuracy than about 5 micra. Furthermore, there is a conspicuous varia-

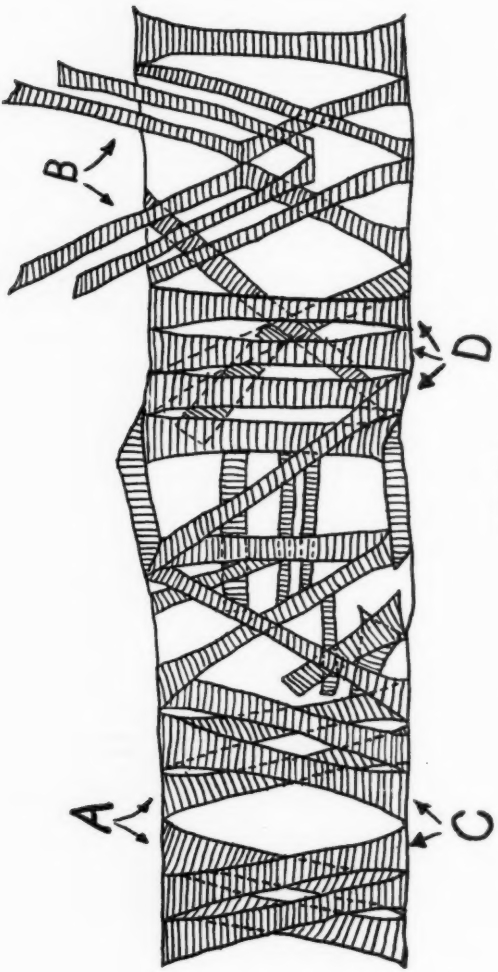

FIGURE 2

Diagram of the muscular system in an abdominal segment of the larva of *Drosophila melanogaster*. A. External dorsal muscle. B. Oblique ventral muscle. C. Internal dorsal muscle. D. Ventral lateral muscle. Only muscles mentioned in the text have been labeled (Modified after Hertwick, 1931).

bility in the material itself resulting from the state of contraction of the fibres, which is not absolutely the same in all instances. Although the method of fixing and killing used relaxed most of the muscles, the equality of relaxation is nevertheless not absolute, as shown by the contraction waves which could be encountered in many cells. It was, however, difficult to count the number of cross bands with exactitude. The cross bands are found primarily in the myofibrils which in our material enjoy a high degree of independence with respect to contraction and relaxation. As a result there was seldom a strictly regular pattern of cross striations such as is found in vertebrate skeletal muscles or in muscles of imaginal stages of insects. Usually an individual fibril could not be followed throughout the entire length of a cell, because the fibrils very often intermingle and cover one another, so that counting had to be continued in neighboring fibrils. Several counts were made in each specimen and the most consistent number recorded. Ten individuals were examined in each group. Table 2 summarizes the results of these measurements and counts.

The figures listed in this table show the following facts. As the total length of the larvae increases during succeeding instars, the dorsal muscle also increases in length. The number of cross bands increases steadily from the shorter to the longer muscles. Dividing the number of cross bands into the length of each muscle the average length of a single sarcomere for this group of muscles is obtained. The important finding is, that this value varies only slightly during the whole period of growth, but stays nearly constant.

The variability of the muscles, as expressed by the coefficient of

TABLE 2

Increase in number of cross bands in the dorsal muscle of larvae with increasing body length. All measurements are in micra. M is the arithmetic mean, s the standard deviation, v the coefficient of variation.

Instar	Body length	Length of the Muscle			Number of bands			Height of Sarcomeres
		M	s	v	M	s	v	
I	1,260	109	8.56	7.77	17.2	0.74	4.37	6.31
II	1,440	123	7.87	5.46	22.4	0.75	3.40	5.54
II	1,980	175	7.20	2.97	28.0	0.94	3.35	6.25
II	2,700	221	6.13	2.72	37.6	1.79	4.71	5.87
III	2,980	272	7.27	2.66	46.0	2.32	5.04	5.91
III	3,400	303	1.46	4.81	51.0	2.17	4.52	5.96
III	4,000	379	1.85	4.89	70.0	1.40	2.25	6.15

variation v is highest in the youngest specimens. This is probably due to experimental conditions. Since the material in the younger stages is extremely delicate, it unavoidably became slightly stretched during mounting, which influenced the length of the muscles. The relatively high degree of variation in the latest stages is very likely due to intrinsic variability of the material which becomes more evident with advancing age. The amount of variability in the number of cross bands is evenly distributed over all age stages and probably due to intrinsic variability. The same is true for the height of the sarcomeres.

3. *The Myofibrils*

In addition to their growth in length the muscle cells also increase in width and thickness. Since it is known that muscle fibres are made up of still smaller fibrous structures commonly referred to as myofibrils, it is obvious that their increase in number and thickness must be responsible for the growth in thickness and width of the entire muscle cell. The study of the myofibrils in our material was obscured by a number of difficulties. The stains commonly employed for demonstrating their presence in vertebrate or adult insect muscle tissue, especially Heidenhain's hematoxylin, failed completely in the younger stages of the larval muscle cells for unknown reasons. Other dyes used by Dempsey, Wislocki, and Singer (1946) stained the whole cytoplasm without any differentiation of fibrils or cross bands. Cross bands become visible with all these stains only in the advanced third instar. The observations on which the following account is based were made in preparations stained with safranin or fast green using polarized light. The muscle cell chiefly examined was the X-shaped oblique ventral muscle of the sixth abdominal segment.

It seems that in our material there are no myofibrils having a definite thickness and being arranged at a definite spacing from one another, such as have been described by a number of histologists for the vertebrate muscle or for muscles of the imaginal insect stages, for instance by Meigs (1928) in the wing muscle of the fly. This condition might be characteristic for adult muscle tissue, but not for cells in larvae with active growth. A very fine fibrous constitution of the cells could be observed when examining the preparation under the oil immersion with polarized light; but the true nature of the fibrils is submicroscopic. What one really perceives are groups or bundles

of these fibrils which vary greatly in width. They range from barely perceptible filaments of less than half a micron to those of several micra. All of these bundles are characterized by having their cross bands approximately at the same level, and by being separated from other such bundles by small interspaces. These interspaces, very small in the youngest stages, become more conspicuous later on, since there seems to be a tendency for the sarcoplasm to increase more rapidly than the filamentous material. For this reason the fibrils are more closely packed in younger than in older cells. Sometimes bundles of fibrils and interspaces form a regular pattern in the older cells and become visible also in ordinary light. Then they show the appearance of myofibrils in the classical sense. This phenomenon is, however, by no means common.

The question arises whether these bundles of submicroscopic fibrils are artefacts or natural units. Apparently many of the interspaces owe their existence, or at least much of their width, to the process of dehydration, when the preparation is passed through the series of alcohols. On the other hand, essentially the same pattern could be seen when the material was studied in the fixing and preserving medium, which was a 10% solution of formol. Furthermore, observations showed in some cases, a sort of fission seemingly initiated along the whole length of the bundles, the parts being still held together at the regions of the Z-lines. Consequently there seemed to be a clustering of submicroscopic units into temporary fibrils or bundles, which are liable to break up into smaller bundles, and these begin again growing in width. The bundles reach approximately the same width in cells of all instars. For this reason it seems feasible to call these bundles myofibrils, keeping, however, in mind that they are not permanent entities, but transitory structures of no constant width and thickness. A width of 1.2 to 1.5 micra is very common.

The reason why these myofibrils break up frequently into smaller bundles can be easily explained. The muscle cells in larvae are attached to a fold in the body wall called the tendon or insertion; not at a single point, as in vertebrates, but along their entire width, so that different fibrils are fastened to different points of the cuticle fold. Now, when the larva grows in diameter the circumference of its body wall also increases, and so do its parts, some of which are tendons to which muscle cells are attached. When the cuticle fold increases in length, the myofibrils are drawn apart. The amount

of stretching of the insertion to which the oblique ventral muscle is attached, at different stages of the larva, is shown in Table 3.

Since not all of the myofibrils in growing cells have the same diameter, it is impossible to give exact and comparable figures for their increase in number. Assuming, however, that they were all of the same width of 1.2 micra, which value can be found in many of them, an equivalent number has been calculated for the ventral muscle in

TABLE 3

Increase in width of insertion and number of myofibrils of the oblique ventral muscle. Figures give the arithmetic mean of 10 observations with standard deviation. All measurements are in micra.

Instar	Body length of larvae	Width of insertions	Number of myofibrils
I	1,260	12.2±1.42	10
II	1,440	18.1±1.98	15
II	1,980	22.3±2.12	19
II	2,520	30.6±0.00	25
III	2,700	36.6±3.27	30
III	3,600	52.3±3.42	43
III	4,140	53.7±4.22	45

larvae of different ages, based on the length of the insertions. The values thus obtained are listed in the fourth column of Table 3. All values of this table are taken from 10 measurements.

4. *The Nuclei*

It was not the intention of the present paper to include the strictly cytological aspect of nuclei in growing cells. Since our interest is limited to the process of cytoplasmic growth, only such observations on nuclei are recorded as were thought to have a bearing on the central problem. The cell selected for this investigation was the central fibre of the lateral muscle. The stages considered were the same as in the preceding sections.

Muscle cells are syncytia, i.e., they contain numerous nuclei. Their number ceases to increase after the larva hatches from the egg. In the lateral muscle the most frequent number is 10, 11 is less frequent, the number 12 occurs occasionally. Of 35 fibres examined 22 (63%) had 10 nuclei, 10 (28%) had 11, and 3 (9%) had 12. The arrangement of the nuclei within the cell is fairly constant. At the end of the first instar they are lined up along the interior side of the fibre

underneath the sarcolemma, which arrangement remains constant. There is a noticeable crowding of the nuclei toward both ends of the cell. In 12 fibres, which had together 126 nuclei, 47 of them were found in the anterior third, 29 in the middle third, and 48 in the posterior third. Observations showed that occasionally two nuclei were located at the point of attachment, side by side, instead of the usual linear arrangement.

The general appearance of the nuclei varies only slightly during development. All of them show an extremely large nucleolus located in the central portion of the nucleus. The rest of the nucleoplasm forms a coarsely granulated network distributed around the nuclear membrane. In the later stages the network becomes more clearly differentiated, and in favorable cases groups of filaments resembling chromosomes could be identified. Nuclei located toward the terminals are usually denser and smaller, while those in the central portion of the fibre are larger, but less dense. The same difference can be observed between the nuclei of younger and older individuals: the former are denser, the latter are less dense. Nuclei in the earlier stages are almost round. They become increasingly elongated with advancing age.

Discussion of Results

1. *Increase in Length of the Muscle Cell*

Since the cytoplasm of muscle cells consists chiefly of myofibrils which extend from one end of the cell to the other, growth of muscle cells reduces itself chiefly to the question of how the myofibrils gain their length during growth. The presence of cross bands in the myofibrils presents the opportunity to make some definite statements. Considering things merely apriori there are several possibilities which seem equally probable. It could be that the sarcomeres were about equal in number in corresponding muscles in larvae of different age, but that they were shorter in the younger ones and longer in the older. That would imply that increase in length of the myofibrils is effected by increase in length of every sarcomere. There is another possibility: It may be that sarcomeres were equal in length in younger and older individuals, but corresponding cells differed in number of sarcomeres in specimens of different age. That would imply that entire sarcomeres were added during the growth of muscle cells. Finally, of course, there is the possibility that both processes worked together,

that entire sarcomeres were added and the sarcomeres themselves were increasing in length.

The figures listed in Table 2 indicate that the possibility enumerated in the second place is the one realized in larvae of *Drosophila*. All of the sarcomeres in younger and older specimens have approximately the same height; their number is steadily increasing as we go from the shorter to the longer muscles. Consequently, the figures prove that growth in the myofibrils, and with it that of the entire muscle cell, is brought about by the addition of entire sarcomeres, which, in turn, have an equal length and do not continue to grow once they have been formed.

As can be seen, the length of a single sarcomere is about 6 micra, which is the usual high value found in insects, and is almost three times as great as that found in frog muscle, where Buchthal (1940) measured 2.18 micra as the average length in resting fibres. The value of 6 micra is characteristic only for muscles serving in locomotion, the sarcomeres in the muscles which manipulate the mouth hook are much smaller.

Figure 3 illustrates the general conclusion. The sarcomeres are as high in the shorter as in the longer muscles, their number, however, increases with increasing length of the entire muscle cell.

2. *The Site of Growth*

Now the question arises as to where the addition of new sarcomeres takes place. Different possibilities could be suggested. It could be that they were added along the entire length of each myofibril, say, by splitting the ones present. On such a hypothesis sarcomeres in the process of splitting and those of all stages of development should be observed. Such is not the case in the material studied. It is true that there are differences in the height of sarcomeres, but they can be readily explained by assuming different states of contraction, because they always occur in groups, and are well known to histologists as fixed contraction waves. Furthermore, it is hard to imagine how the uniform pattern of cross striations extending sometimes throughout the entire width of the cell and resulting from the juxtaposition of the cross bands of all the single myofibrils, could be explained if many sarcomeres were at different stages of proliferation and development. Finally, as has been pointed out by many histologists and physiologists, every sarcomere with its isotropic and

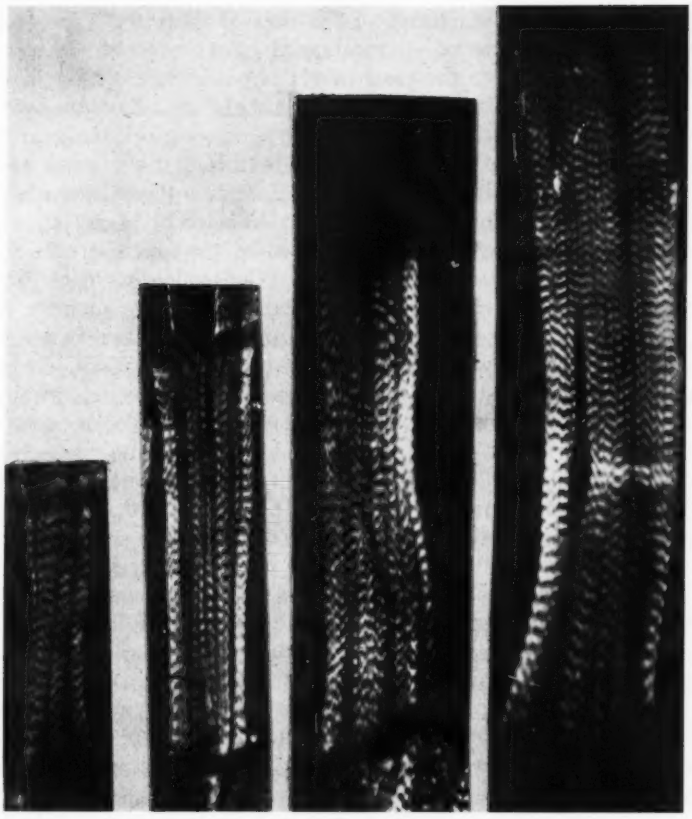

FIGURE 3

The external dorsal muscle of the sixth abdominal segment in larvae of different ages, in polarized light, at the same magnification X 130. The original length of these fibres is: a. 0.266 mm.; b. 0.400 mm.; c. 0.665 mm.; d. 0.800 mm. Note the increase in number of sarcomeres.

anisotropic region is a functional unit. It is not likely that such a complex structure could divide without a profound reorganization, for which there is no evidence.

The remaining possibility is that new sarcomeres are continually added at certain points, very probably at the extremities. No experimental proof can be offered at the present time that the sarco-

meres are really manufactured at the ends of the cells. There are, however, two instances of circumstantial evidence which should be considered here. When the cross bands of older muscle cells are examined more closely, it can be found that those of the middle portion have a markedly more differentiated appearance than those at the ends. Examination of very young cells reveals that the cross bands are still in a state of indifferentiation. This finding can be explained by taking into account the fact, known from embryology of vertebrates, that the process of differentiation of the sarcomeres is still going on after they have been formed. Consequently, more differentiated sarcomeres are older and the undifferentiated younger. But the latter are found precisely at the ends of the fibres, therefore, it can be concluded that they are also produced here at the ends.

The second circumstantial evidence lies in the following consideration. Caspersson's work (1950) has made it probable that protein synthesis is carried out in the nucleoli, at least in its initial steps. The primary material for the synthesis of the large protein molecules accumulates temporarily in the large nucleoli, which are characteristic for growing cells. It can be expected that the position of the nuclei will be at points where most of the new material is needed. It has been noted above that the nuclei are more numerous at the extremities of the muscle fibres. This distribution of the nuclei can be understood surmising that they are situated at points where growth is proceeding. The fact that protoplasmic fibrils in muscle cells are growing by apposition at the end points and not by intussusception along their entire length is in contrast to the mechanism of growth of microfibrils in cell walls of plant cells, advanced recently by Frey-Wyssling (1948).

3. *The General Principle of Growth*

If we limit our attention to that phase of growth which implies increase in size alone, there seems to be a certain contradiction involved in it. Every organism is an extremely complex system, and at the first view it seems impossible to enlarge such a system without destroying its unity. The contradiction becomes still more evident when we consider that the organism has to carry out all its manifold functions while it is being enlarged. Nature solved this problem by composing the organism of numerous subunits, namely the cells. The organism gains in size by the multiplication of its subunits by cell

division. During this process some of the cells cease functioning temporarily and divide, while the majority of the cells preserve their normal activity.

The same principle can be observed in growing muscle cells. They also consist of numerous functional subunits, namely the sarcomeres. While the cell as a whole keeps functioning new subunits are continually being built up and assume their function as soon as they are mature. There is, however, this great difference at the subcellular level, that the subunits are not produced by division here, as is the case in cell multiplication.

It would seem, furthermore, that the principle of growth by increasing the number of functional subunits can be found also in cytoplasmic growth in general. It is known that the fundamental activities of the cytoplasm, such as respiration, synthesis of glandular secretion, and the like, are dependent upon the activities of certain enzymes, which, in turn, are grouped into microscopic or submicroscopic granules or corpuscles. They need not necessarily be all of the same kind. The point is that there are always very numerous particles of the same kind in a particular type of cytoplasm, and that these particles are the functional subunits by which the activity of the cytoplasm as a whole is accomplished. It is easy to see that an increase in number of these functional subunits by a mechanism which is still unknown, necessarily leads to an increase in amount of the cytoplasm of the cell, and can be accomplished while the cell as a whole is engaged in its specific functions. Similar considerations can be applied to the decrease of the amount of cytoplasm which takes place every time when the cytoplasmic portion of the cell is divided, after the nucleus has undergone mitosis.

SUMMARY

Whole mounts of larval skins of larvae of *Drosophila melanogaster* to which the muscles were still attached, were studied by following a small number of them through succeeding stages of growth.

The examination of the external dorsal muscle revealed that the number of sarcomeres is steadily increasing, and that the sarcomeres of different age stages have approximately the same height. Consequently entire sarcomeres are continually added along the myofibrils.

The myofibrils are bundles of submicroscopic fibrils. They have no permanent existence, but are split and built up again. The

reason for the splitting is the increase in width of the insertion to which the muscles are attached over their entire width.

The nuclei possess large nucleoli. They are arranged along the long side of the fibres and are more numerous toward the points of attachment.

The new sarcomeres are probably manufactured at the ends of the cells.

Conceiving the sarcomeres as the functional subunits of the muscle cell, the principle of growth in these cells was found to be the addition of functional subunits. This principle is likely to be operating also in the cytoplasmic growth of every cell.

References

1. Alpatov, W. W. 1929. Growth and variation of the larva of *Drosophila melanogaster*. *Jour. Exp. Zoöl.*, **52**, 407-432.
2. Buchthal, F. 1940. Experiments on the minute structure and function of the striated muscle fibre. *Acta Psychiatr. et Neur.* **15**, 46-68.
3. Caspersson, T. 1950. Cell Growth and Cell Function. New York: Norton. 185 pp.
4. Dempsey, E. W., Wislocki, G. B., & Singer, M. 1946. Some observations on the chemical cytology of striated muscle. *Anat. Rec.* **96**, 221-243.
5. Frey-Wyssling, A. 1948. The growth in surface of plant cell walls: Submicroscopic and physiological aspects. *Growth* **12** (Suppl.), 151-169.
6. Hammett, F. S. 1942. The rôle of amino acids and nucleic acid components in developmental growth. *Growth* **6**, 59-125.
7. Heidenhain, M. 1919. Ueber die Noniusfelder der Muskelfaser. *Anat. Hefte I* **56**, 323-402.
8. Hertweck, H. 1931. Anatomie und Variabilitaet des Nervensystems in *Drosophila melanogaster*. *Zeitschr. wiss. Zoöl.* **139**, 559-663.
9. Kurnick, N. B., & Ris, H. 1948. A new stain mixture: Acetoorcein-fast-green. *Stain Technol.* **23**, 17-18.
10. Lewis, W. H. 1939. Some contributions of tissue culture to development and growth. *Growth* **3** (Suppl. I), 1-14.
11. Meigs, E. B. 1928. Striated and smooth muscle. Special Cytology, Ed. by E. V. Cowdry, New York, Hoeber.
12. Weismann, A. 1864. Die nachembryonale Entwicklung der Musciden. *Zeitschr. wiss. Zoöl.* **14**, 189-336.
13. Wilson, L. P. 1939. The influence of sulfhydryl and sulfoxide on the length growth of first instar larvae in *Drosophila melanogaster*. *Growth* **3**, 409-417.

Growth, 1950, **14**, 295-325.

DIFFERENTIATION IN A CALLUS CULTURE OF SEQUOIA SEMPERVIRENS

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The advantages of the use of the sterile culture method for study of differentiation in the tissues of vascular plants have not been fully utilized. A piece of tissue may be removed from the original plant and given nearly constant environmental conditions. The isolation may be considered to remove the inoculum from "organismal" influences of the original plant body, and thereby presumably exclude this factor in its subsequent growth. Some idea of the nature of differentiation, whether it is cellular or organismal, may thus be obtained. In addition, the environment may be varied and the effects, if any, of this factor on the process of differentiation may be noted.

Materials and Methods.—Thick green shoots from a burl (Fig. 1) were used. Except for the presence of great amounts of parenchyma in pith and cortex, these stems were normal anatomically (Fig. 2). One-centimeter-long portions of these shoots were surface-sterilized with 5% sodium hypochlorite, washed in sterile distilled water, and the lower ends were pushed half-way down in agar slants of culture medium. The latter was Knop's x ½ according to Nobécourt (1937) and Gautheret (1942) with 3% sucrose, indoleacetic acid at 1×10^{-6} by weight, and thoroughly washed Japanese agar at 1.5%. The cultures were started in Pyrex glass test tubes 22 mm. x 220 mm. In this culture medium during the course of a month both the basal and apical ends of the stem piece formed callus (Fig. 3). The placing of 5 ml. of sterile distilled water in the bottom of the slant maintained a high humidity in the air of the tubes above the agar. Without this added water it was found that the agar quickly shrank and callus growth of the stem pieces was suppressed in the dry winter air of the laboratory. This technique was obtained from Nobécourt (1937) who added aqueous culture medium to the bottom of agar slants.

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FIGURES 1-3

Fig. 1. Sprouted burl with the thick green shoots that were used as inocula for the origin of callus. x 1/5. Fig. 2. Transverse section of a thick green shoot such as was used as inocula for the origin of callus. x 28. Fig. 3. Stem segment after 24 days in culture medium, showing basal and apical calli. x 4.

After a month's growth, pieces of callus were excised from the bottoms of the stems and placed on the agar of new tubes of the culture medium. Growth has been continuous. Transfers are made at two-month intervals. The cultures are now in the sixteenth transfer. After the third transfer the callus attained such great volume that it was difficult to remove it from the tubes. Pyrex test tubes of larger diameter, 36 mm. x 190 mm. have therefore been subsequently used.

Previous to using, all glassware was scrubbed with calgon and soap, rinsed in hot tap water, drained dry, internal surfaces covered with a cleaning solution of 10% HNO_3-90% H_2SO_4, rinsed twice in hot tap water, drained, rinsed once in distilled water, and drained dry. The water used in the medium was pyrex-distilled. Chemicals used were all of reagent grade.

Most of the cultures have been grown in a constant temperature (21°C) constant humidity (60% relative humidity) room. They have been exposed to diffuse daylight from the north side of this room and they develop sufficient chlorophyll to give them a light green color. In this favorable humidity the agar does not dry out and the callus grows well. Agar slants, with added water at the bottom are therefore no longer used and the agar surface is allowed to solidify at right angles to the axis of the tube.

Little attempt has yet been made to modify the original components of the culture medium. Three per cent sucrose appears to be the optimum concentration, since one per cent and five per cent of this sugar have given poor growth of callus. Growth appears to be more rapid with indoleacetic acid at 10^{-6} than at 10^{-7}.

Origin of the Callus.—Median longitudinal sections of the stem-pieces after a month's growth in the agar showed that the upper callus was brought about by activity of the vascular cambium (Fig. 4). This meristem had become continuous below the upper cut surface with the sides of the stem and had produced secondary wood in amounts sufficient to cause an apical sweliing. The activity of vascular cambium here can be compared with that in callus formation on the upper cut ends of sterile-cultured stems of *Vitis Coignetiae* Pl. shown in Fig. 4 of Morel (1948). Gautheret's (1945) Fig. 4 shows a similar action of the vascular cambium in the lower cut surface of a carrot root. These are very interesting cases of change in polarity of the vascular cambium and may be cited as exceptions to

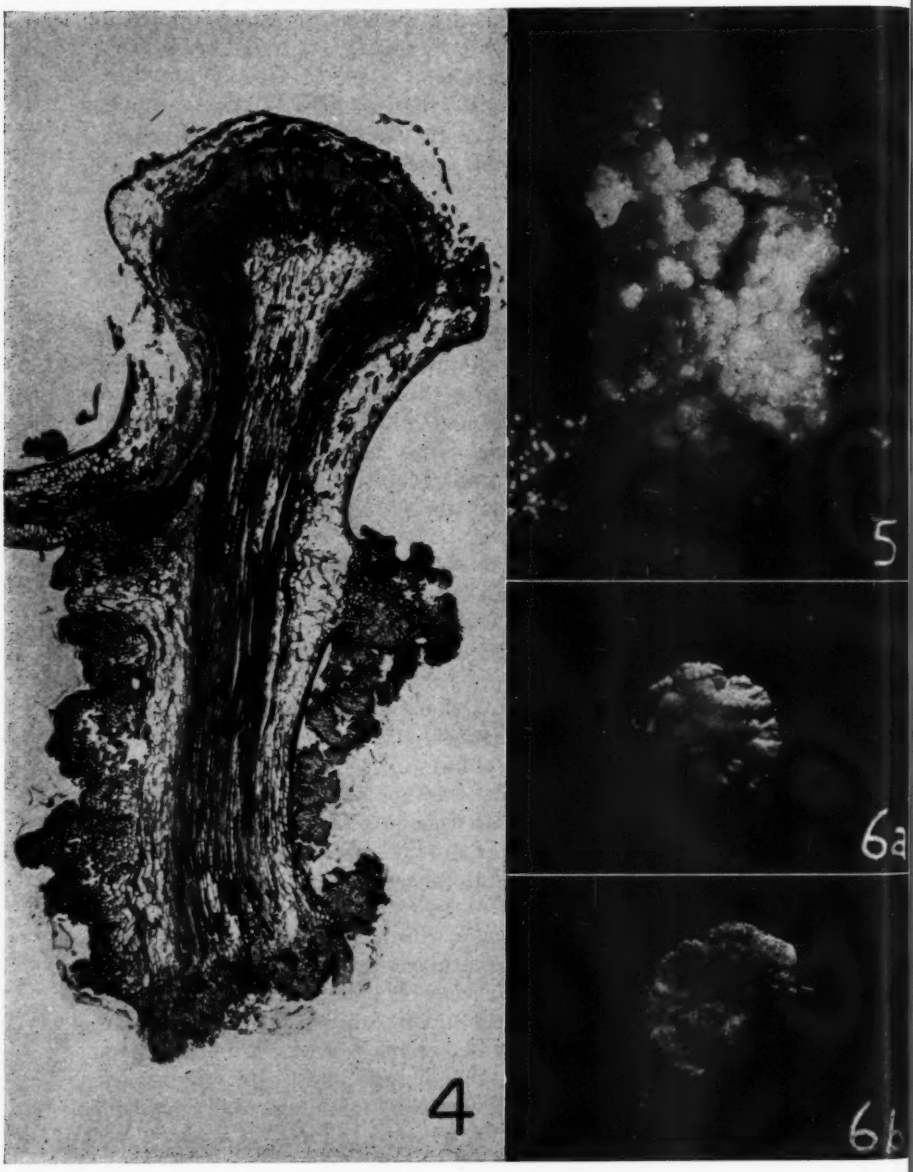
Bloch's (1943, p. 288) statement that attempts to change the general organ polarity have met with little success, apart from local changes. The callus developed on the lower end of the stem-piece of *Sequoia* came almost entirely from proliferation of the cells of the cortex (Fig. 4). Segretain (1941) found that new tissues could proliferate either from the cortex or the bare cambium of tobacco stem-pieces in culture.

Anatomy of the Callus in Sterile Culture.—All callus cultures grown were of firm consistency, with little air space between the cells, and though formed largely of parenchyma, gave noticeable resistance to a knife when they were cut up preliminary to transfers.

During the first three transfers the callus cultures were as thick as they were wide (Fig. 5). With subsequent transfers the callus formed a layer that extended over the surface of the agar (Fig. 6a and b). The layer was in intimate contact with the agar only at its edges; the center was usually in the air. A cross-section of the callus usually gave the form of an inverted cup. Morel (1948) described a similar form of callus for *Malva sylvestris* (Pl. 12 and p. 71). Gautheret (1948) described the form of culture of *Salix* Caprea during the first passages as approximately spherical; later it manifested a tendency to spread over the surface of the agar to give a sort of green crust of fragile digitations.

Irregular growth prevailed in most of the calli examined by serial sections. Most of the tissue consisted of thin-walled parenchyma, but there were occasional masses of thick-walled tracheids of irregular shape (Fig. 7). Often the tracheid-masses were surrounded by a cambium which had produced phloem-like cells externally. In addition to the formation of new cells around the tracheid masses, often the edges of the callus had a phellogen-like meristem that produced long files of parenchymatous cells (Fig. 10, 7m). These marginal meristems appeared to contribute the major number of new cells during the growth of the callus.

In three calli of ten from the third transfer that were killed, dehydrated, embedded in paraffin and sectioned serially, there were vascular formations that closely resembled steles (Figs. 8, 9). These steles were in some cases discontinuous when serial sections of the callus were followed (Figure 9 shows the end of one of the steles). In the other seven calli of this sample, and in all calli sampled from subsequent transfers, no such vascular formations were found in the sections.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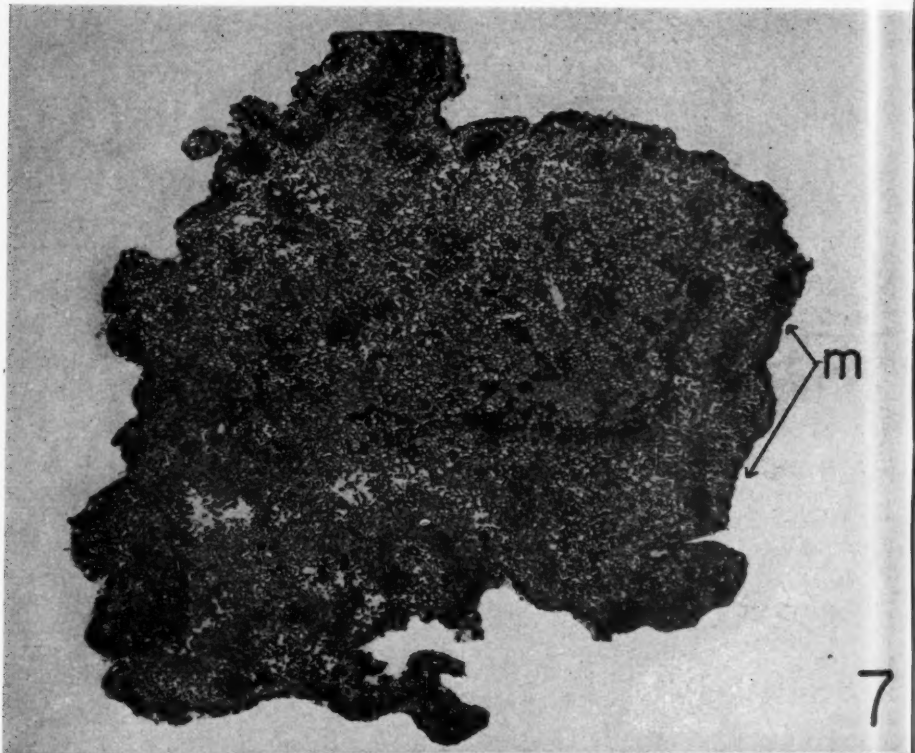
FIGURES 4-6

Fig. 4. Longitudinal section of a stem segment after 53 days in culture. Apical callus has originated through the activity of the vascular cambium that became continuous across the cut end of the stem; a spherical nodule of secondary wood has been produced. Basal callus arose almost entirely through the proliferation of the cortex. x 10. Fig. 5. A callus culture at inoculation time for the third transfer. The form is thick and approximately isodiametric. x 4. Fig. 6a. View of callus at thirteenth transfer showing the form of extended crust over the agar. x 1. Fig. 6b. View of the lower side of the same callus showing the margins of the crust that were in intimate contact with the agar, leaving the central portion in the air above the agar. x 1.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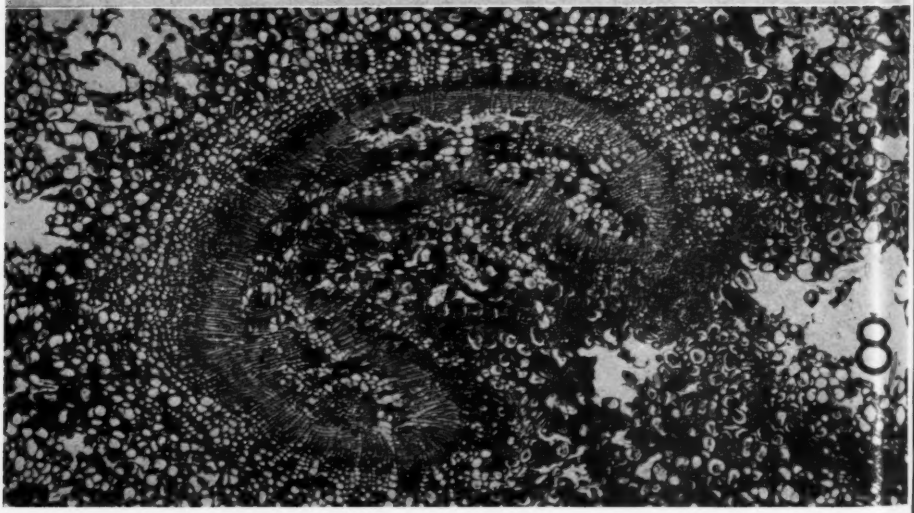
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FIGURES 7-8

Fig. 7. Section of a callus after the fifth transfer. Numerous tannined cells, as single individuals and in groups are present throughout the callus. Many tracheid-masses are present. Much of the margins are occupied by phellogen-like meristem, as at m. x 9.5. Fig. 8. Section of a portion of a callus at inoculation time for the third transfer, showing a stele. x 27.

All sections of the calli showed numerous tannin-filled cells among the clear parenchymatous cells. The proportion of tannined cells to clear ones appeared to be constant and approximately the same as that found in the cortex and pith of the stems that had been used as the inoculum. This appearance has been noted in sections of the calli from the twelfth transfer after which the cultures had grown more than two years isolated from the original stems. No restriction of the tannined cells to the old portions of the callus was noted. This contrasts to Gautheret's (1948) description of the tanniferous cells of the cultured callus of *Salix Caprea,* about which he wrote that they were localized to the aged regions where proliferation is slowed down. In the callus of *Sequoia* the tannined cells appeared to undergo divisions at the same rate as the clear cells. The frequent appearance of long files of cambium-like cells on the margins of these calli indicate that the "initials" of such chains may be either clear or tannined cells (Fig. 10). Similar appearances may be seen in Morel's (1948) Plate 7 showing files of clear and tannined parenchymatous cells from a vascular cambium in a culture of tissues of *Vitis* and his figure of the vascular cambium around a xylem group in a culture of tissues of *Crataegus monogyna.* Bloch (1948) described long files of tannined cells in internodes of *Ricinus communis;* they arose by divisions of a tannined initial cell in the intercalary rib meristem immediately behind the shoot apex. He suggested that the tannined cells are of a permanent nature and represent a cytoplasmic transformation. The finding of numerous cells with only small amounts of tannins where the formation of new cells occurs (Figs. 10, tp; 11, tp; 14, tp) can be interpreted to mean that these cells were losing their tannin.

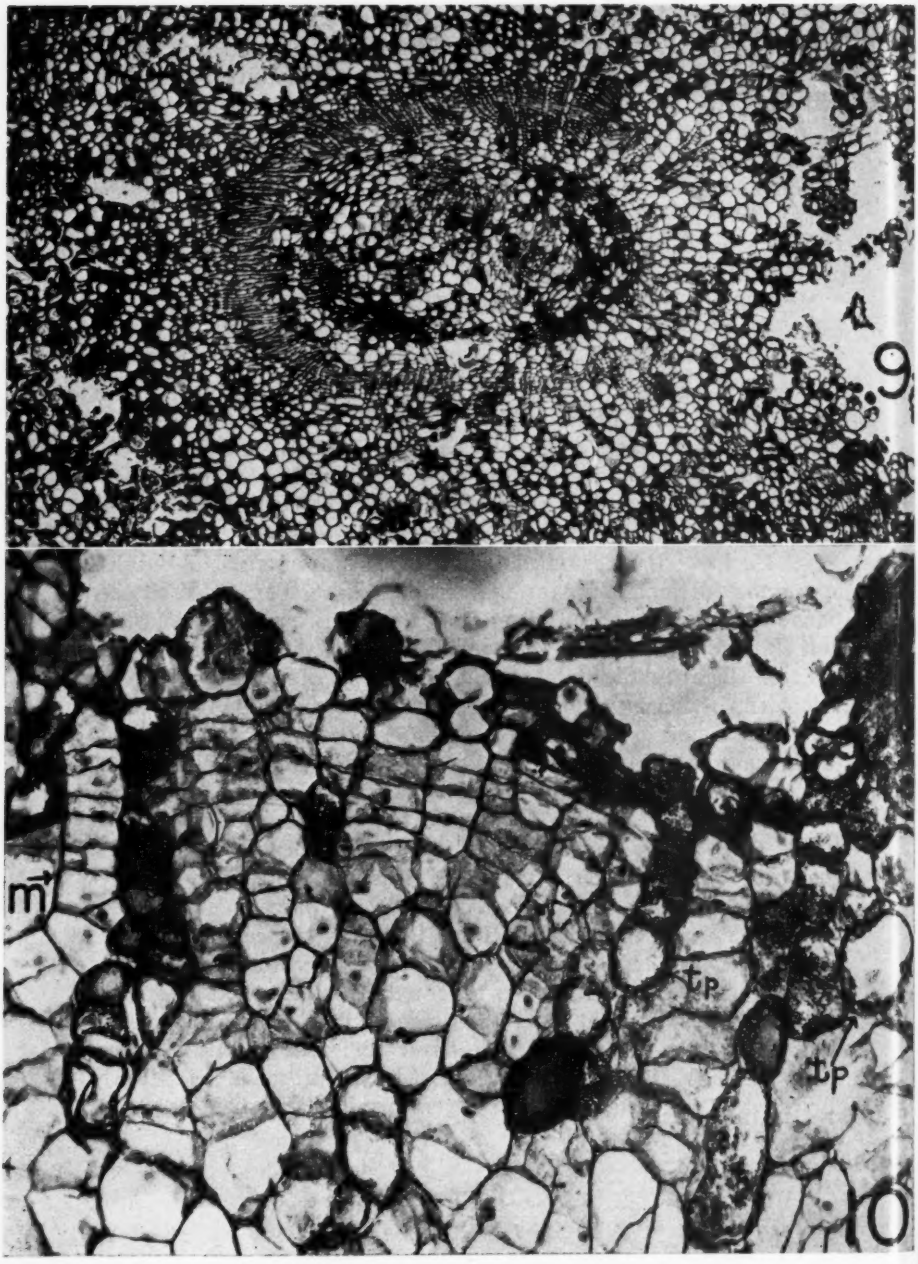
The tannins were studied in fresh sections by treatment with various reagents suggested by Tunmann (1931). Brown precipitates were formed after treatment with $FeSO_4$. Blue-black precipitates were formed after treatment with $FeCl_3$. Methylene blue formed dark blue precipitates. Concentrated vanillin followed by concentrated HCl produced light brown granules or deep red precipitates; some cells were almost black. $K_2Cr_2O_7$ produced light brown granules or a uniform dark brown precipitate in the cells.

The masses of tracheids appear to usually originate from the divisions of a single clear parenchymatous cell (Fig. 11, a, b, c) and the lignification of some of the central derivatives (Fig. 12). In only

a few instances has a tannined cell been found divided in such fashion (Fig. 11, d). During the later stages of development of the tracheid mass, if a cambium is formed around it, the adjacent tannined and clear cells may be incorporated in this meristem and divide alike to produce derivative cells that add to the group (Fig. 13). A similar incorporation of clear and tannined parenchymatous cells into a cambium is shown in Morel's (1948) Fig. 23 depicting a xylem group of *Crataegus monogyna* from a callus culture. The clear and tannined derivative cells of *Sequoia* may differentiate to tracheids around the center of the mass (Fig. 14).

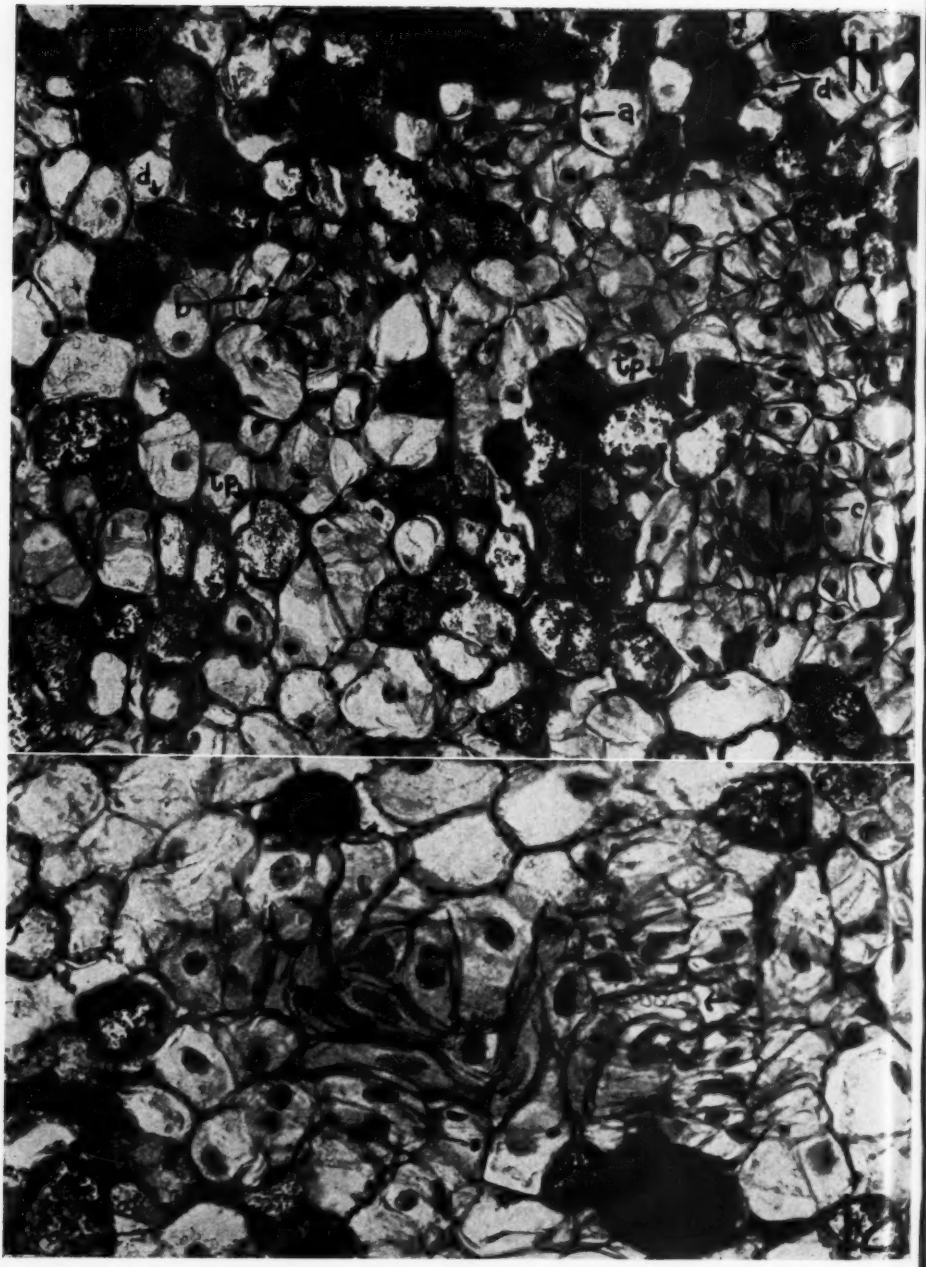
Numerous stages of formation of buds have been observed in the calli. By studying this regeneration in calli grown after the third to fifth transfers, the process was followed in parenchyma devoid of any normal vascular system; the only vascular tissue present was the occasional nests of tracheids. The early stages of the regeneration of a shoot apex appear to be from the divisions of several clear parenchymatous cells (Fig. 15). Tannined cells may be incorporated in the edges of the meristem, but not in the center. The tannin content appears to be very small in some cells of the meristem (Fig. 15, tp). It is assumed that such cells are stages in the disappearance of tannins in the regenerating shoot apex, since all sections made of older shoot apices from the callus entirely lacked tannins. Probably the tannins were resorbed during the dedifferentiation.

Later stages in the development of the shoot apex show the development of foliar primordia and a flat meristem (Fig. 16). Older buds were found to contain a cone-shaped shoot apex not unlike that of the normal shoot (Figs. 17, 19). The cells of the shoot apices were in groups surrounded by an original wall (Fig. 18) resembling those of *Lupinus* (Ball, 1949). At the base of the shoot apex in the young stages of its formation a mass of small, densely cytoplasmic cells was observed (Figs. 17, 18). This was apparently the beginning of procambium; under older buds the procambium had developed into a long subjacent tract that was suspended from the shoot apex and ended blindly in the parenchymatous cells of the callus (Figs. 19, 20). There was never a differentiation of the procambium toward a tracheid nest; there appeared to be no relationship between the pre-existing vascular tissue and that produced underneath the buds. Basipetal differentiation of this procambium is clearly indicated. Tannined cells were never found in the shoot apices of


9
m
tp
tp
10

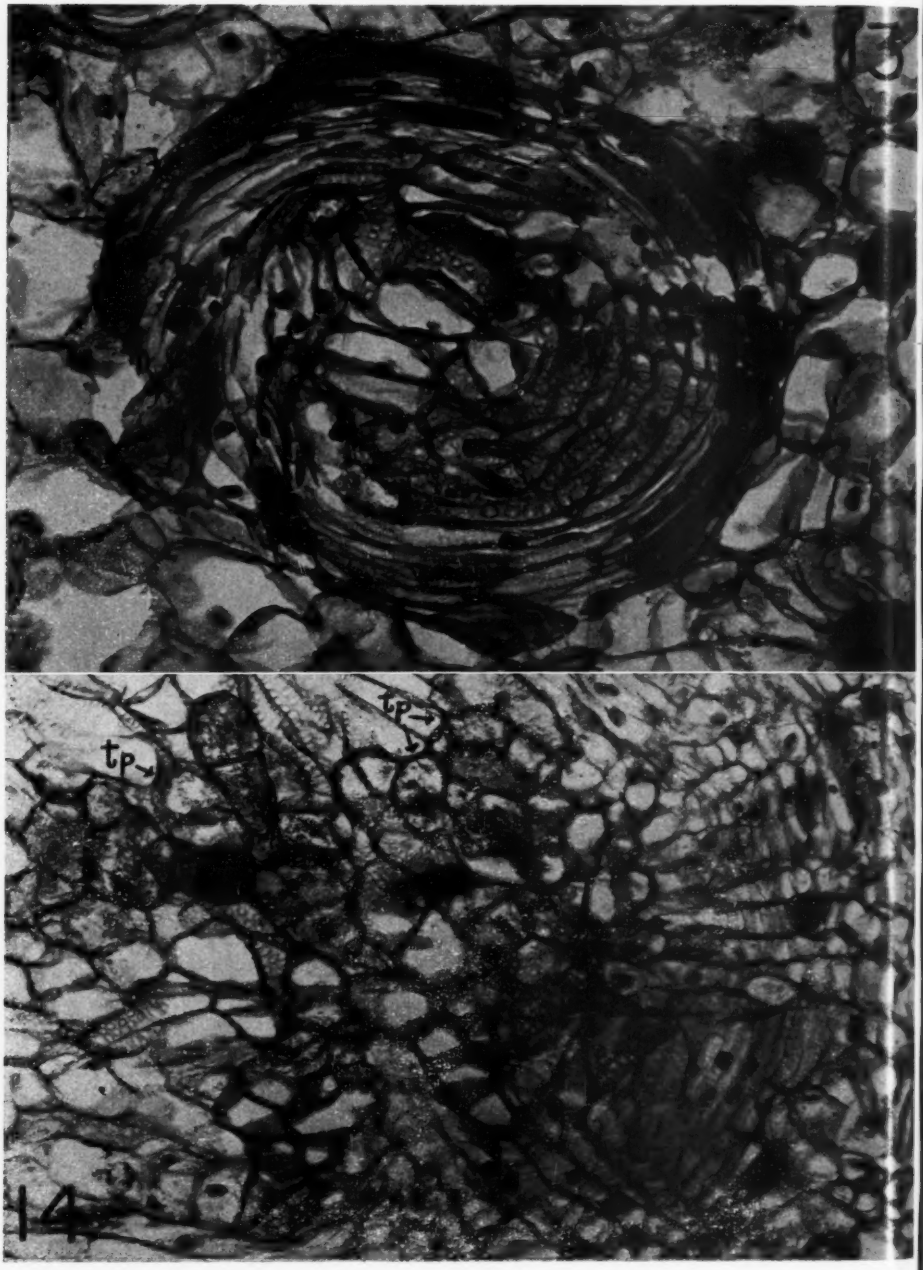
FIGURES 9-10

Fig. 9. Section of a portion of the callus of Fig. 8, approximately 600μ below that level showing the attenuation of the stele; above and below this level the stele was much better developed—about as in Fig. 8. x 27. Fig. 10. Section of a marginal meristem of the callus, showing the files of cells produced by the phellogen-like meristem, m. The files consist of clear cells, of tannined cells, or mixtures of the two. Some of the tannined cells, tp, have a very low content of tannin. x 170.


a
d
d
b
tp
tp
c

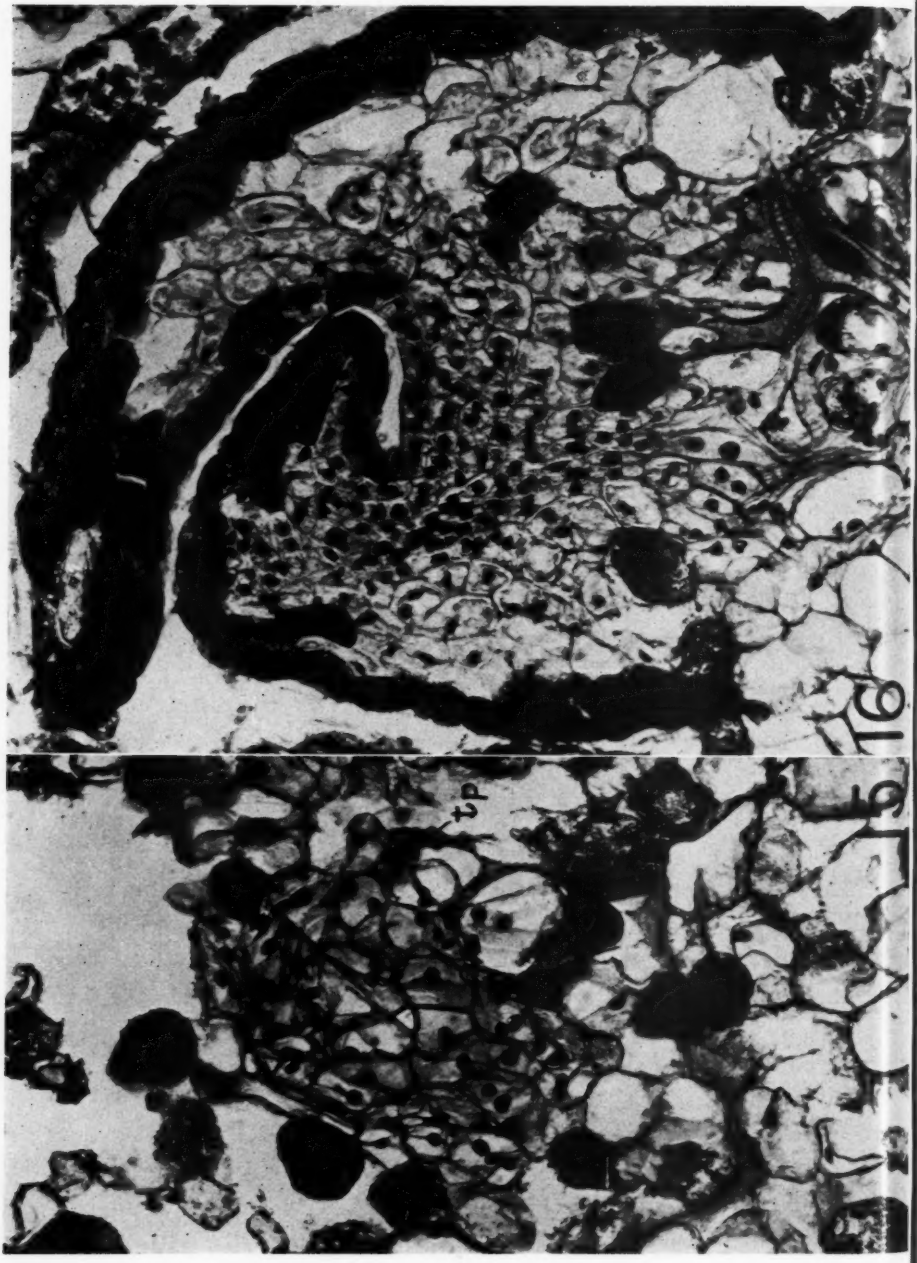
FIGURES 11-12

Fig. 11. Section of a callus after the fifth transfer showing at a, b, and c stages in the divisions of single clear cells to begin the formation of tracheid-masses. Rarely, tannined cells were found to have divided in this fashion, as at d. Several examples, tp, are seen of cells containing only small amounts of tannin. x 170. Fig. 12. Section of the same callus showing an advanced stage in the development of a tracheid-mass. An internal cell has become a tracheid, at arrow. x 280.


3
tp→
tp→
14

FIGURES 13-14

Fig. 13. Tracheid-mass in callus after the fifth transfer. A cambium surrounds the central wood. The cambium has incorporated clear and tannined cells into itself; initials of each type have divided producing derivatives like themselves. x 280. Fig. 14. Portion of a tracheid-mass surrounded by a cambium in a callus after the twelfth transfer. Cells containing only small amounts of tannin, tp, near the center of the mass were probably in the process of differentiation into tracheids. x 194.


tp
tp

FIGURES 15-16

Fig. 15. Section of callus after the fifth transfer showing a young stage of regeneration of a shoot apex. Divisions have occurred in a group of clear cells to produce a small meristem. Tannined cells have been incorporated in the edges, and one cell, tp, that contains only a small amount of tannins was probably in the process of losing its tannins. x 194. Fig. 16. Longitudinal section of a bud from a callus after the fifth transfer. The shoot apex is composed entirely of clear cells, while tannined cells occur only in subjacent regions and in the young leaves. At this early stage of development, the shoot apex is a flat meristem. x 194.

these older buds, but were only found in older tissues of leaves, cortex and pith.

No roots have ever been regenerated from any of the callus cultures, which have now been carried through sixteen transfers at intervals of two months.

Since the production of buds occurred frequently in the calli examined, it can be assumed that the relatively high concentration of growth regulator in the medium did not prevent the formation and growth of buds. Skoog and Tsui's (1948) suggestion that the effect of auxin on bud inhibition is dependent on the concentration of auxin, not per se, but in proportion to the concentration of other components of a "reaction system" has not been tested with regard to the callus of *Sequoia*. Nobécourt (1947) noted the spontaneous origin of buds in the callus of carrot; if one assumes that the culture medium had the same content of indoleacetic acid as that described in his paper of 1937, then these buds arose in a high concentration of auxin.

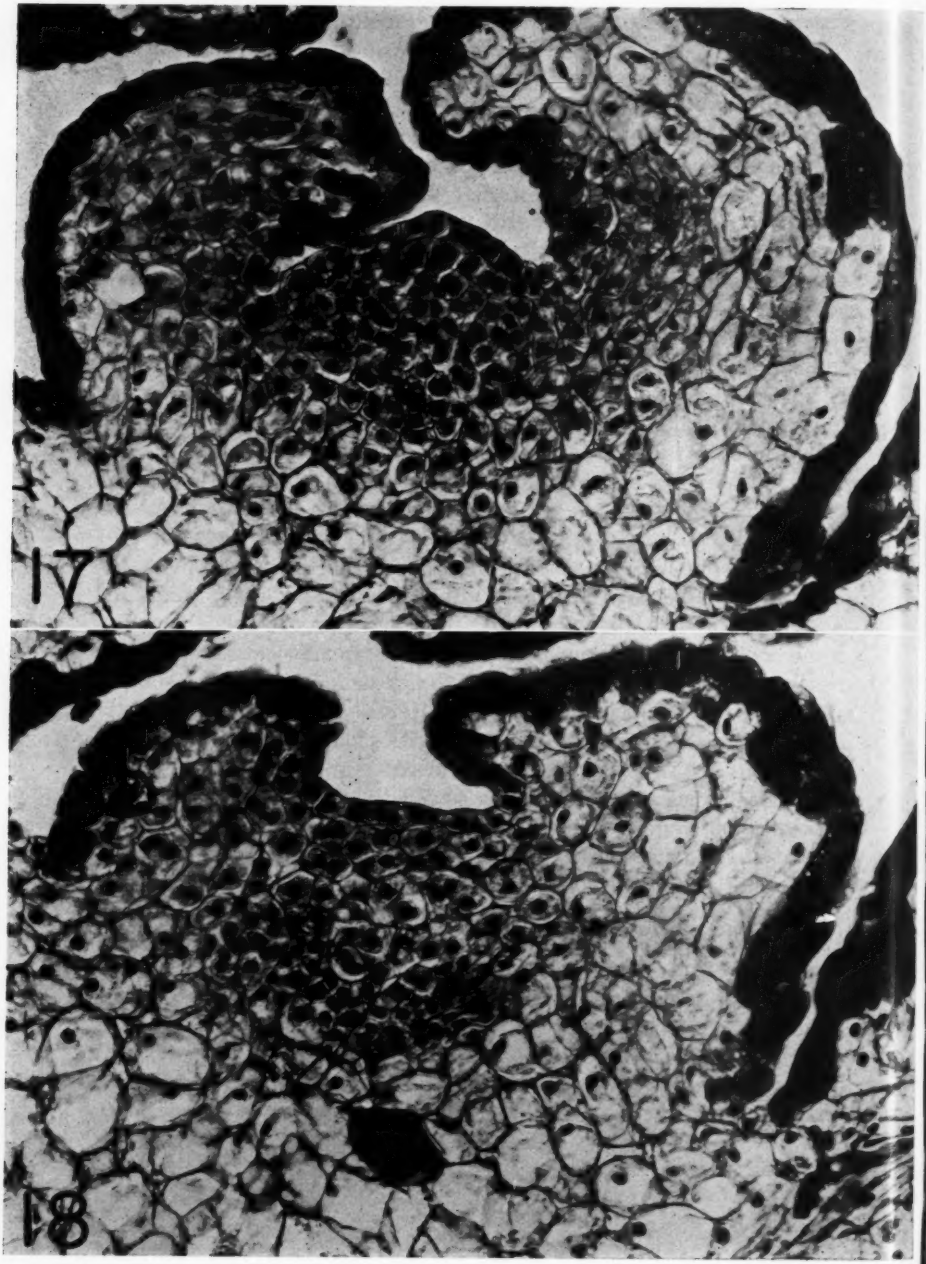
Discussion.—The origin, growth requirements and anatomy of the callus culture of *Sequoia sempervirens* are not unlike those described for dicotyledonous plants by Nobécourt (1937), Gautheret (1942), Morel (1944).

There appears to have been a relationship between the thick, massive callus formed during the first three transfers and the presence of the steles. These large vascular formations appeared to have no conductive function, since the callus usually had no shoots and never any roots. Furthermore, the steles were in some cases discontinuous; this has been taken to indicate their lack of function in conduction. The occurrence of these vascular formations is very diffiult to explain. As one possible cause it may be suggested that there has been a persistence of the growth pattern of the woody stem through the first three transfers. With the fourth and subsequent transfers, this pattern was lost with the loss of the thick callus form and the assumption of the spread-out form. Another theory to account for the presence of the steles is that they are pathological manifestations in a callus. Küster (1925) recorded their presence in the calli formed in woody plants subjected to wounding.

The presence of tannined cells in the original stem and their persistence in the callus makes it possible to follow the growth relations of these cells under controlled conditions. The permanence of these cells in the cultures through fifteen transfers may be taken as evi-

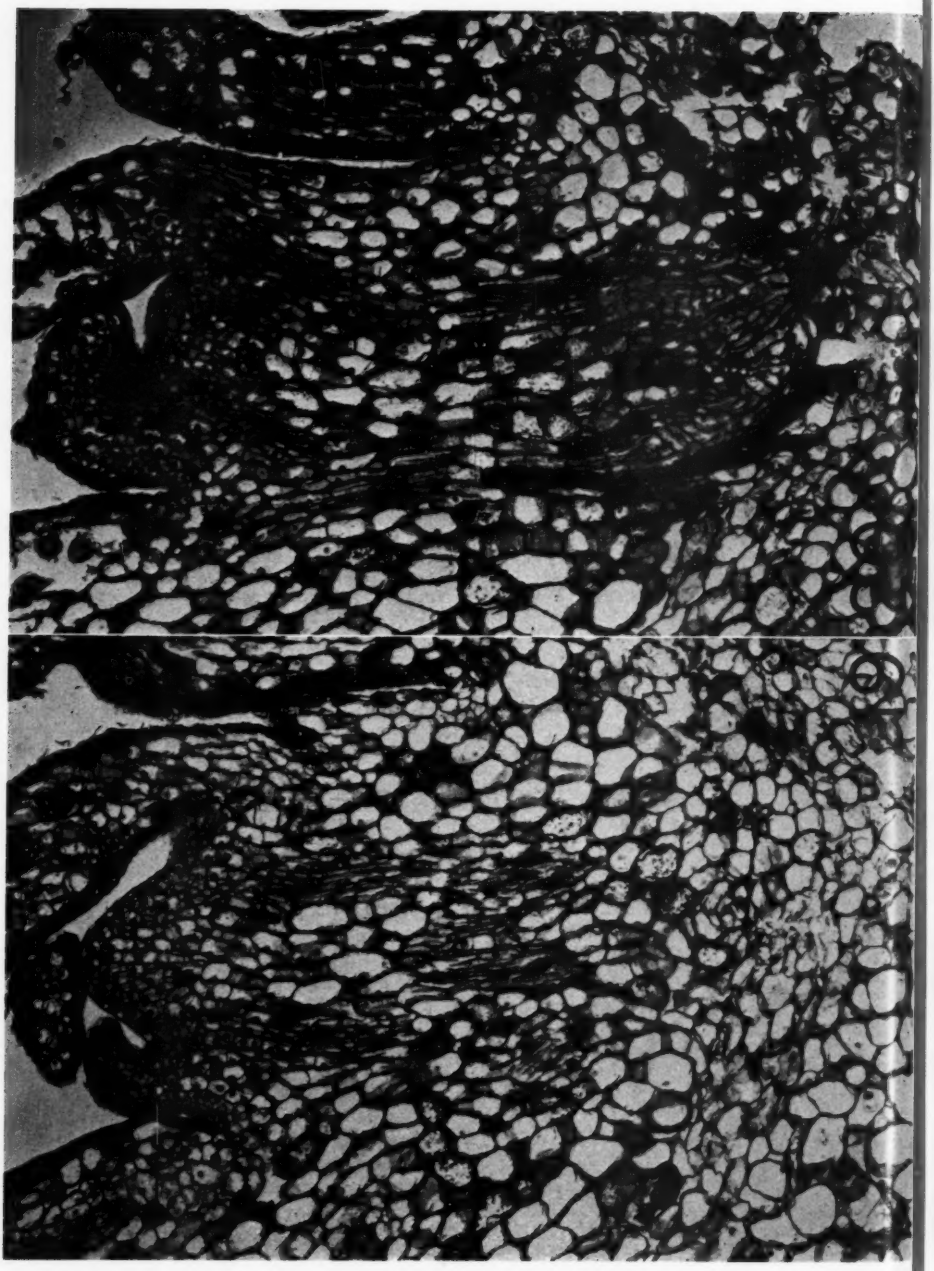
dence that a tannined cell transmits the qualities of tannin-formation to its descendants through an indefinite time, or at least, that the physiological conditions in the cultured callus are sufficiently close to those of the original plant to permit formation and storage of tannins in certain cells. Bloch's (1948) idea that these tanniferous cells are the result of permanent cytoplasmic changes may not be strictly true for the callus of *Sequoia,* for here the cells apparently lost their tannins as they were incorporated into regenerating shoot apices, into tracheids, and as parts of the files from marginal meristems. The tannin-forming character does not appear to be an irreversible one in these cells. This character may be compared with that of cystolith initials in certain plants. Wells (1916) showed that the cystolith was inhibited or prevented entirely from developing in hackberry leaf galls caused by *Pachypsylla mamma.* Meyer (1944) showed that the cystolith initials in leaf epidermis of *Urtica dioica* in ostioles of galls caused by *Perrisia Urticae* might divide anticlinally to produce two cystoliths. If division was periclinal, the inner derivative was an ordinary parenchymatous cell while the outer one became a cystolith. In other cases the cystolith development was suppressed in the gall.

The question may be asked: does the presence of tannin in the cell constitute differentiation of a sort that restricts its growth activities as compared with the clear cell? The growth of the callus gives some indications that the tannined cells may have less potentiality for growth and differentiation than the clear cells. In the formation of the groups of tracheids, the original divisions usually occur in the clear cells, seldom in tannined cells. Divisions of the latter do not usually form groups of the small, meristematic cells that originate the tracheid-masses. Many of the tracheid-masses grow by the formation of a cambium around the mass of wood. The cambium appears to encompass those cells that happen to occur next to the wood cells; clear cells or tannined cells may be incorporated in this meristem. Each type of cambial initial produces cells like itself, and apparently the derivative cells, whether clear or tannined, may differentiate into tracheids or phloem. The formation of long files of cells at the margins of the callus was frequently seen in the serial sections. This superficial cambium-like growth appeared to encompass all of the cells, whether tannined or clear, within its area. If the initial was clear, a file of clear derivative cells resulted. If the initial was tannined, a file of tannined cells resulted. In these long files of cells, it was fre-


17
18

FIGURES 17-18

Fig 17. Longitudinal section of a bud (from callus after fifth transfer) showing the median view of the shoot apex which is in an advanced stage of development; the meristem here is a cone. The shoot apex consists entirely of clear cells. The group of small, densely-cytoplasmic cells (arrow) at the base of the shoot apex is apparently the beginnings of procambium. x 194. Fig. 18. Off-median section of the shoot apex of the same bud. The putative procambium at the base of the shoot apex (arrow) is clearly shown. The groups of cells surrounded by an original wall in the shoot apex are easily seen. x 194.



FIGURES 19-20

Fig. 19. Median longitudinal section of the shoot apex of a bud in the callus after the third transfer. The shoot apex is a dome and consists entirely of clear cells; tannined cells occur only in subjacent regions and in the young leaves. The subjacent procambium, though not shown in median section, is well developed and extends several millimeters beneath the shoot apex. x 65. Fig. 20. Off-median longitudinal section of the shoot apex of the bud shown in preceding figure. This section shows clearly the subjacent, suspended procambial strand which ends blindly in the callus beneath the shoot apex. x 65.

quently observed that a row of cells might be partially tannined and partially clear, and that some cells had a very small amount of tannin. Such occurrences could either come about by the production of tannined cells by clear initials, the formation of clear cells from tannined initials, or the loss of tannins by an individual cell. Some doubt may therefore be expressed about the permanence of the character of tannin-formation in the derivatives of such cells.

The origin of buds by divisions of the clear cells and never by the tannined cells may be taken as evidence that the latter have a lower potentiality in regeneration than the former. The shoot apices of these buds do not have tannined cells. Tannined cells, however, are produced a short distance behind the shoot apex in cortex, pith and in leaves. The lack of tannined cells in the regenerated shoot apices of this callus, and the occurrence of numerous tannined cells in the subjacent maturing tissues is in keeping with both the writer's observations of these facts in apices of normal shoots and with Sterling's (1945) work on apices of this plant. His Figures 2, 3, 5, 9 and 10 (p. 120) show shoot apices entirely without tannin and there are numerous tannined cells in the subjacent pith and cortex and in the young leaves. Craft's (1943) Figures 2, 3, 4, 5 show the same thing. Other illustrations in the literature show similar restriction of tannined cells to the regions subjacent to the shoot apex: *Pseudotsuga taxifolia* (Sterling, 1946); *Abies venusta,* where the tannined cells reach within three or four cell layers of the tip of the apex (Foster, 1939); healthy buds of apricot and peach (Reed, 1941).

In the study of regeneration of shoot apices from callus the tannined cells incorporated in the meristem appeared to lose their tannin. It would seem that the presence of tannin is incompatible with the normal activity of cells in a shoot apex. This interpretation is strengthened by Reed's (1941) observation that shoot apices of peach and apricot trees suffering with zinc deficiency may have tannins in their cells (see his Figures 2 and 5). Zirkle (1932) found abundant tannins in the cells of the shoot apex of the dwarf shoot of *Pinus strobus,* presumably after that meristem had produced its leaves and was in a non-functional state. In contrast, he found no tannins in cells of the shoot apex of the long shoot of the same plant, presumably when that meristem was in the condition of producing new cells and tissues. In the root tips of *Pinus strobus* he found tannins in the peripheral

zones; the only cells free from tannins were the meristematic initials, the central cylinder and a core piercing the root cap and extending from the periblem to the apex.

Many studies of tannins in plants have given good evidence that the tannins are never moved out of the cells in which they were originally formed (Sachs, 1859, 1862, 1865). Other studies have shown that the total tannin content of plants may change greatly during a growing season. For example, Stitt and Clarke (1941) recorded that the tannin content of *Lespedeza* leaves increased until June 30 and then gradually decreased until September 22. The first and last leaf samples contained less than half as much tannin as the mid-season ones. In the absence of cytological studies, it is possible to interpret these studies as merely varying tannin content of different leaves. Huber's (1929) findings that the cotyledons of *Quercus ballota* decreased 30% in tannin content during germination can only be interpreted as the moving from individual cells of their tannin content. Mascré (1919) showed the dissolution of the "corpuscles tannoïdiques" in the tapetal cells of *Datura arborea* during pollen formation. The ergastic cells (presumably tannined) beneath the shoot apex of *Pseudotsuga taxifolia* lose their deep-staining contents during expansion of the apex and other cells acquire that type of content (Sterling, 1946).

In *Sequoia,* as well as in the other cases cited, it is obvious that the cells of the shoot apex have in them the potentiality of producing tannined cells as well as clear cells, since all cells of the shoot owe their origin to this meristem. It follows, therefore, that the clear parenchymatous cells have in them the potentiality of producing tannined derivatives.

The lack of tannined cells in the shoot apex, and the occurrence of such cells in the cambium can be taken as an evidence of difference between the metabolism of the primary and secondary meristems. Buvat (1944) pointed out numerous cytological and structural differences between cells of primary and secondary meristems during regeneration of callus to form stems and roots. He described the lack of tannins, for example, in growing root apices of tomato and their presence in cells of arrested lateral root primordia.

The presence of tannins in certain cells, their absence in others, their disappearance during the regeneration of shoot apices, are merely the morphological end results of metabolism in the tissues. Studies of

this metabolism should yield information concerning the processes of differentiation and dedifferentiation.

The production of a subjacent vascular strand by these buds is a clear case of basipetal differentiation under the influence of the shoot apex. There could be no question of the influence of pre-existing vascular tissues, since the callus contained none except the isolated tracheid groups. This experiment is therefore a clear demonstration of the differentiative effect upon parenchymatous cells by buds. It may be considered a confirmation and extension of the works of Camus (1949) where the buds, either regenerated or grafted into fleshy roots in culture caused a subjacent basipetal differentiation of vascular tissues toward and inside the pre-existing vascular cylinder. In Camus' work the possibility was not excluded that the already-present vascular tissue of the fleshy root was influencing the buds, either regenerated, or added by grafting, to carry on their differentiative effects.

The origin of a shoot apex from the divisions of a number of parenchymatous cells may be taken to indicate that this meristem is necessarily multicellular in origin. This observation can be compared with the experimental work of dividing the shoot apex of *Lupinus albus* into several parts; the smallest subdivision of an apex that would regenerate a new apex was one-sixth of the original. Smaller subdivisions failed to regenerate (Ball, 1951). Since the bud may be considered a new individual or organism, its origin from a group of cells may be compared with the recent work in cancer research that indicates that there must be a minimum number of aberrant cells to constitute the beginnings of a tumor that escapes the growth controls of the parent organism (see Hollomon and Fisher, 1950). Buvat (1944) also described the origin of the bud from callus of endive by the dedifferentiation of a very circumscribed little group of cells.

The cambium-like meristems of the margins of the calli, around the masses of tracheids, and in the steles, when they occur, may be considered differentiation on the multicellular, or organismal level. The formation of masses of meristematic cells by the divisions of several clear parenchymatous cells to form a shoot apex may likewise be considered an organismal differentiation. Here cells divide according to a field of activity; if a cell happens to be the zone of activity it divides to produce cells that contribute to the general structure.

The formation of tannined cells or clear cells, and the formation of the tracheid-masses may be considered differentiation on the level of the individual cells. This differentiation appears to not be a function of the organism but of the single cell.

Summary

Cultures of callus were obtained from thick green shoots of a burl. Growth appeared to be due mainly to the activity of marginal meristems, although tracheid-masses were often surrounded by cambial layers. The proportion of tannined cells in the callus was approximately the same as found in cortex and pith of original stem. The cambia at the margins of the callus or around the tracheid-masses incorporated both clear cells as well as tannined cells, and either type could function as initials to produce a file of clear or of tannined cells. Occasional cells contained small amounts of tannin; it is suggested that these cells were in the process of losing tannin. The tracheid-masses arose by the divisions of single clear cells to form a mass of small cells; some of the central cells became lignified and lost their protoplasts. Rarely, tannined cells were found to have divided in a manner resembling the origin of tracheid masses. Tannined derivative cells of the surrounding cambium were observed to undergo lignification and loss of protoplast, including tannin, in the differentiation to tracheids. Shoot apices originated by the divisions of small groups of clear cells, although tannined cells could be incorporated in the edges of the meristem. Tannined cells thus included in a regenerating shoot apex were observed to lose their tannins. The functional shoot apex of a bud in the callus, as well as in a normal shoot, lacked tannins. It is suggested that the presence of tannin is incompatible with the normal function of the shoot apex, a primary meristem, and that this characteristic is one that may distinguish it from the cambium, a secondary meristem. Procambium occurred at the base of the shoot apices and differentiated basipetally into the parenchyma of the callus, where it ended blindly. The tannined cells probably have less potentiality in regeneration than the clear cells. The cambia and the shoot apices may be considered organismal differentiation within the callus. The formation of tannined cells, clear cells, tracheids, may be considered cellular differentiation.

References

BALL, E. 1949. The shoot apex and normal plant of *Lupinus albus* L., bases for experimental morphology. *Amer. Jour. Bot.* **36**, 440-454.

————. 1951. Experimental division of the shoot apex of *Lupinus albus* L. Growth and Differentiation in Plants, Monograph of the American Society of Plant Physiologists. In Press.

BLOCH, R. 1943. Polarity in plants. *Bot. Rev.* **9**, 261-310.

————. 1948. The development of the secretory cells of *Ricinus* and the problem of cellular differentiation. *Growth* **12**, 271-284.

BUVAT, R. 1944-45. Recherches sur la dédifférenciation des cellules végétales. *Ann. Sci. Nat.: Bot. 11e sér. 5 & 6*, 1-228.

CAMUS, G. Recherches sur le rôle des bourgeons dans les phénomènes de morphogénèse. *Rev. de Cyt. et de Biol.* **9**, 1-199.

CRAFTS, A. S. 1943. Vascular differentiation in the shoot apex of *Sequoia sempervirens*. *Amer. Jour. Bot.* **30**, 110-121.

FOSTER, A. S. 1939. Structure and growth of the shoot apex of *Cycas revoluta*. *Amer. Jour. Bot.* **26**, 372-385.

GAUTHERET, R.-J. 1942. Manuel technique de culture des tissus végétaux. Masson et Cie., Paris.

————. 1945. La culture des tissus végétaux. Comptes rendus du Congrès de Paris 1945 (64e Sess., t. III) de l'Assoc. fr. pour l'Avan. des Sci., 204-226.

————. 1948. Sur la culture indéfinie des tissus de Salix Caprea. *C.R. Soc. Biol. Paris* **142**, 807.

HOLLOMON, J. H., & FISHER, J. C. 1950. Nucleation and growth of cell colonies. *Science* **111**, 489-491.

HUBER, HANNA. 1929. Über den Zustand und die Rolle der Gerbstoffe in der Pflanze. *Jahrb. wiss. Bot.* **70**, 278-327.

KÜSTER, E. 1925. Pathologische Pflanzenanatomie. Dritte Aufl. Jena, Gustav Fischer.

MASCRÉ, M. 1919. Sur le rôle de l'assise nourricière du pollen. *C.R. Acad. Sci. Paris* **168**, 1120-1122.

MEYER, J. 1944. Observations sur l'évolution pathologique et la ségrégation précoce des cellules cystolithiques de l'ortie dioïque. *Rev. cyt. et cytophys.* **7**, 218-221.

MOREL, G. 1944. Sur le développement des tissus de vigne cultivés in vitro. *C.R. Soc Biol. Paris* **138**, 62.

————. 1948. Recherches sur la culture associée de parasites obligatoires et de tissus végétaux. *Annales des Épiphyties* **14** (N.S.), 1-112.

NOBÉCOURT, P. 1937. Cultures en série de tissus végétaux sur milieu artificiel. *C.R. Acad. Sci. Paris* **205**, 521-523.

————. 1947. Apparition de pousses feuillées sur des cultures de tissus de carotte. *C.R. Soc. Biol. Paris* **141**, 590.

REED, H. S. 1941. Effects of zinc deficiency on cells of vegetative buds. *Amer. Jour. Bot.* **28**, 10-17.

SACHS, J. 1859. Physiologische Untersuchungen über die Keimung der Schminkbohne (*Phaseolus multiflorus*). *Sitzungsber. d. kais. Akad. d. Wiss. Wien*, **37**, 57.

————. 1862. Zur Keimungsgeschichte der Dattel. *Bot. Zeitung* **20**, 241-246, 249-252.

————. 1865. Handbuch der Experimental-Physiologie der Pflanzen. Leipzig.

SEGRETAIN, G. 1941. Formation de cals en milieu aseptique à partir de fragments de tiges de tabac. *C.R. Soc. Biol. Paris* **135**, 648.

SKOOG, F., & TSUI, C. Chemical control of growth and bud formation in tobacco stem segments and callus cultured in vitro. *Amer. Jour. Bot.* **35**, 782-787.

STERLING, C. 1945. Growth and vascular development in the shoot apex of *Sequoia sempervirens* (Lamb.) Endl. I. Structure and growth of the shoot apex. *Amer. Jour. Bot.* **32**, 118-126.

———. 1946. Organization of the shoot of Pseudotsuga taxifolia (Lamb.) Britt. I. Structure of the shoot apex. *Amer. Jour. Bot.* **33**, 742-750.

STITT, R. E., & CLARKE, I. D. 1941. The relation of tannin content of sericea lespedeza to season. *Jour. Amer. Soc. Agron.* **33**, 739-742.

TUNMANN, O. 1931. Pflanzenmikrochemie. Gebrüder Borntraeger, Berlin.

WELLS, B. W. The comparative morphology of the zoocedia of *Celtis occidentalis*. Ohio. *Jour. Sci.* **16**, 249-290.

ZIRKLE, C. 1932. Vacuoles in primary meristems. *Zeits. f. Zellforsch. u. mik. Anat.* **16**, 26-47.

Growth, 1950, **14**, 327-339.

THE EFFECT OF LOW TEMPERATURE ON *AMBLYSTOMA PUNCTATUM* IN RELATION TO DEVELOPMENTAL ACCELERATION[1]

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Inhibition of developing larvae or embryos is a natural occurrence in the life cycles of various groups of animals. Such a phenomenon is seen in the diapause of insects, in the dormant periods of some crustaceans and in the winter eggs of rotifers.

Blocking of development can be effected experimentally and subsequently the organism will recover from the block to pursue its developmental pattern, Moran ('25); Rapkine ('31); Clowes and Krahl ('34); Tyler and Scheer ('37).

Acceleration of development subsequent to low temperature treatments has been recorded in recent literature. Insects placed in low temperature environments for a part of their developmental period and returned to higher temperature showed an increase in rate of development, Bodine ('25); Bliss ('26); Parker ('30); Williams ('46). Stockard ('21) observed this phenomenon in *Fundulus*. Buchanan ('38, '40) and Ryan ('41) showed that amphibian larvae inhibited by low temperature are accelerated in their development after removal to higher temperature. Differentiation rate of embryonic thyroid tissue exposed to low and then to higher temperatures has been reported by Carpenter ('45).

The present communication attempts to answer the following questions: Have previous observations purporting to show an accelerated developmental progress following inhibition in Amphibia a basis in fact? Are external evidences of developmental progress in embryos after low temperature treatment corroborated by histogenetic evidences? Is the developmental progress of the different organ systems typical subsequent to low temperature inhibition?

[1]I wish to express my thanks to Professor J. W. Buchanan for suggesting this problem, and my gratitude for his kindness and generosity in its direction.

Materials and Methods

Masses of *Amblystoma punctatum* eggs were used. The eggs were placed in thermos jugs which were filled with water of the pond from which they were removed and were shipped to Chicago by air express on the day on which they were collected.

In a typical experiment each egg was separated from the large jelly mass and observed under a binocular microscope to determine the extent of its development. The eggs were subsequently separated into groups according to their respective stages (Harrison).

From groups of eggs at a given stage, for example, Stage 9, a number of eggs never exceeding 12 were placed in 250 ml. flasks filled with normal tap water from Lake Michigan. This water had been previously chilled to 3°C. in a refrigerator. The flasks containing the eggs were placed in the refrigerator. In all there were 9 flasks of eggs which were prepared in this manner. These eggs are collectively referred to as Group B. From the original egg masses other eggs were placed in 250 ml. flasks. The water in these flasks was previously warmed to 21°C. The flasks were submerged in a water bath, the temperature of which was kept constant at 21°C. ($\pm 0.1°$). There were 12 flasks of eggs prepared in this manner and these shall be referred to as Group A.

Three days after the previous stock of eggs was received other egg masses were received and the eggs were immediately removed from the jelly mass and staged. The eggs were in the same stage as those of Groups A and B at the time the latter were received. They were placed in 250 ml. flasks which contained water previously warmed to 21°C.; these in turn were placed in the bath maintained at 21°C. Five flasks were prepared; they constitute Group X in this typical experiment. Group X, therefore, contains eggs which are 72 hours younger than those in Groups A and B.

The eggs of Group B in the refrigerator were kept at the low temperature for exactly 72 hours. They were then pipetted out and placed in watch glasses containing water at 21°C. Each was observed under a binocular microscope to determine if developmental changes had occurred. They were then transferred to 250 ml. flasks which contained water at 21°C., and were placed in the constant temperature bath. These eggs were of the same age and from the same lots as those in Group A, but their apparent stage of development was the same as that of the eggs of Group X.

When the animals in Group A reached Stage 46 according to the staging of Harrison, the treatment of all the larvae was concluded. This is a distinct end point because at this time the balancers are lost.

A series of finger bowls were filled with chloretone at a strength of 1/3,000% by weight. The larvae were placed in the chloretone according to their respective groups. One by one they were accurately staged, and each one placed in a small vial filled with Bouin's fixing fluid. The specimens remained in the fixing fluid for 24 hours, after which they were washed with a saturated solution of lithium carbonate in 70% alcohol.

Procedures usual to embedding embryological material were used. The animals were sectioned at 10 micra so that some sections were cross, some were frontal and some were sagittal. The larvae were stained with Harris acid haemalum and were counterstained with a saturated solution of Orange G in 100% alcohol.

Results

There were three groups of eggs in these experiments: Group A containing the controls; Group B containing eggs of the same chronological age as Group A, but due to low temperature inhibition were retarded in development for 72 hours; Group X containing eggs deposited three days later than those in Groups A and B, and which were treated in the same manner as the controls.

Observations of the whole larvae were made as they passed through the various stages leading to the end point of the experiments, namely at the time the control animals reached Stage 46. However, no data on these various stages are reported in this paper. Previous workers have amply demonstrated that in the development of amphibians the rate of development is rapid in the early stages with a leveling off at later stages, Atlas ('35); Buchanan ('38); Price ('40); Ryan ('41).

Observations of the developmental progress in these experiments show that the animals in Groups A and X have a rate of development which follows the general pattern described by the workers mentioned above. Initial growth and differentiation is rapid up to a given time and then proceeds more slowly. The larvae of Group B, inhibited by low temperature and returned to a higher temperature also follow the general pattern for amphibians except that the initial growth and differentiation is more rapid than that of Groups A and X and the deceleration is not as sharp.

Studies of sectioned larvae from Groups A, B and X were made for the purpose of determining whether or not developmental acceleration was uniform throughout the entire organism. In analyzing the various organ systems within the larvae it was necessary to choose criteria which would give evidence of such distinct changes that differences were obvious within given systems. The use of the brain tissue and neural tube, for example, as a criterion to show a more rapid development does not give sufficiently fine critical end points to reveal acceleration. The differentiated brains in both control and inhibited larvae appear alike. On this basis the inhibited larvae have certainly overtaken the controls. Yet, nothing is really established, since animals of Group X which are actually three days younger than the controls of Group A have brain differentiation similar to that of the more advanced controls. Furthermore, the brain being a relatively massive structure, a study of its superficial appearance does not reveal distinct end points useful for the quantitative analysis necessary to study acceleration.

In contrast to the brain, the mesonephros allows for accurate distinctions to be made. In the larvae of Group A at Stage 46 the formation of the mesonephros is distinctly advanced. The mesonephros occupies a position to the right and left of the dorsal median line, developing retroperitoneally. The primary mesonephric bodies and their tubular outgrowths are well developed in their respective segments. However, within each segment the secondary mesonephric bodies and their tubules have made their appearance, developing in relation to the primary bodies. By reason of this extensive secondary development and the appearance of the mesonephric tubules the clear-cut metameric arrangement of the mesonephros primordium has almost completely disappeared (Fig. 1). In the caudal region of the developing mesonephros some metamerism is still evident. The primary mesonephric bodies in this region are smaller than those located more anteriorly.

Larvae of Group X which are actually 72 hours younger than those of Group A presented a completely different picture of mesonephric development. In some of the larvae there was an indication of primary mesonephric body formation within a few segments. The general pattern observed in these forms is that the mesonephric cells arrange themselves in position along the pathway of the pronephric duct. Consequently, in these larvae there is no evidence of a well developed mesonephros (Fig. 2).

The low-temperature inhibited larvae of Group B have their mesonephros in a condition intermediate between the two previously mentioned. In these forms the mesonephros shows a definite metamerism, the primary mesonephric bodies being well formed within their respective segments (Fig. 3). Furthermore, the secondary tubular growth is limited to only a few anterior segments. There is no interruption of metamerism by reason of secondary mesonephric bodies and their tubules.

Analysis of these three groups shows that the larvae of Group X which are actually 72 hours younger than the larvae of Groups A and B do not have a well developed mesonephros. Animals in Group B which are physiologically 72 hours younger than the larvae of Group A have their mesonephros developed more extensively than Group X. Mesonephric development of the larvae in Group B approached more closely to that of the larvae of Group A. Hence, if larvae inhibited for 72 hours by low temperature and then returned to a higher temperature did not achieve a developmental rate greater than that which is normal for the control temperature of 21°C. used in these experiments, their mesonephros would be similar to the mesonephros of the larvae of Group X. However, the acceleration is not sufficient to bring the development of the mesonephros to the stage shown by the larvae of Group A.

Larvae from the control Group A show a well differentiated digestive tract. The long pharynx opens into a short tube, the esophagus. The stomach appears as a widened, elongate sac, and differentiation within the stomach is well defined. The mucosal layer has been formed and the cylindrical epithelial cells of the layer have their nuclei basally located. The layers of musculature are not evident, although with careful observation strands of muscle can be seen invading the stomach wall. Finally, the inner wall of the stomach is evaginated into finger-like projections, the primordial rugae. These structures are not completely evaginated, but they do give an outline of the folds of an adult stomach wall (Fig. 4). The proximal end of the intestine is well differentiated. Immediately caudad to the stomach it courses ventrally and then bends craniad toward the liver. At the level of the liver it turns caudally and continues as a straight tube to the posterior part of the trunk region where it opens into the cloaca. The mucosal lining of the anterior division of the intestine has differentiated and the inner layer of the tube has evaginated as well

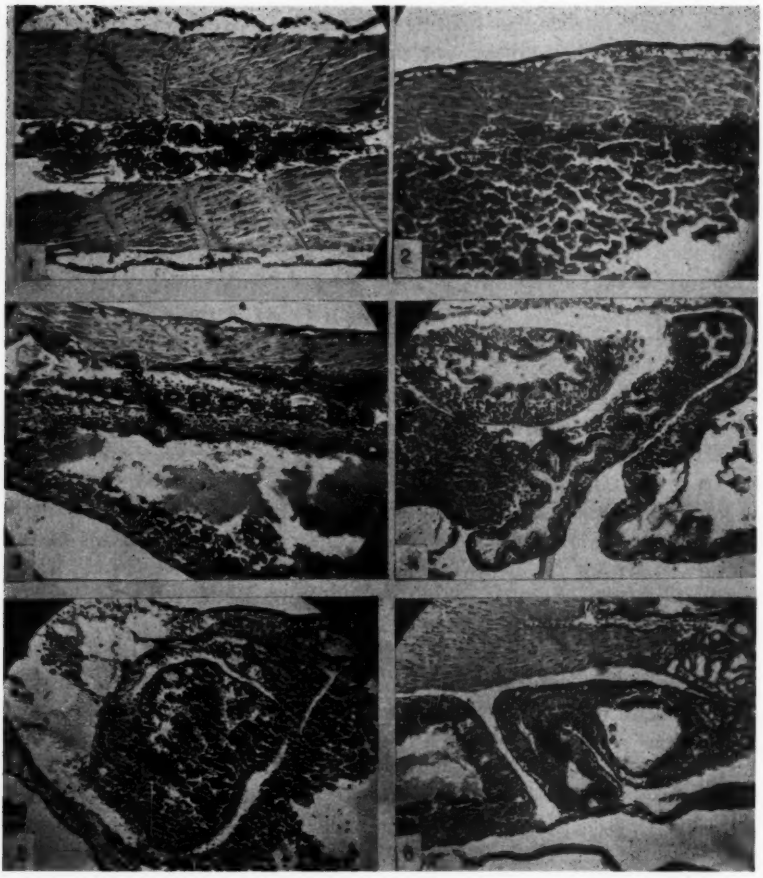

PLATE 1

Fig. 1. Mesonephros of control animal showing loss of metamerism along mesonephros axis due to presence of secondary mesonephric bodies and tubules (Frontal section).

Fig. 2. Mesonephros primordium of 72-hour younger animal of Group X. Primordium being formed between myotomes and yolk mass (Sagittal section).

Fig. 3. Mesonephros of cold treated larva of Group B. The primary mesonephric bodies are segmentally arranged just dorsal to the wall of the intestine (Sagittal section).

Fig. 4. Stomach and intestine of control larva of Group A showing differentiation in both structures. Stomach mucosa appears thrown into small folds as does the intestinal mucosa (Sagittal section).

Fig. 5. Stomach of 72-hour younger larva of Group X which gives no evidence of any mucosal differentiation (Sagittal section).

Fig. 6. Stomach and intestine of cold treated larva of Group B. The stomach lies underneath the pronephros and its mucosal lining is well defined. Note proximal part of intestine which is assuming the shape of a tube (Sagittal section).

formed villi (Fig. 4). The mid-intestine, although not well differentiated is nearly free of yolk material. The wall of this part of the tract is defined and there is a wide lumen.

Differentiation within the digestive tract of the animals belonging to Group X is not extensive. The stomach has developed to the extent that it is recognizable as an elongate sac, but there is no differentiation of its inner layers nor is there evidence of primordial rugae (Fig. 5). In these larvae there is no well defined intestine. Differentiation within the intestine has not taken place except for the region of the cloaca.

The study of the digestive tract of the low-temperature inhibited larvae of Group B reveals this to be intermediate between Groups A and X. The pharynx terminates posteriorly into a short, narrow esophagus. This in turn empties into a stomach. In these forms the stomach has become somewhat elongate as in the controls. The interior of this organ shows to some degree the formation of a mucosal layer but no stomach folds (Fig. 6). A lumen is definitely present, containing traces of yolk material. Caudad to the stomach the intestine turns ventrally as observed in the animals of Group A. This portion of the intestine is strikingly different from the remainder. It takes on the appearance of a definite tube with a lumen free from yolk material, and is bounded by a thick wall (Fig. 6). The inner border of the wall shows no mucosal differentiation nor is it thrown into folds. The remainder of the intestine as far caudad as the cloaca is like the intestine of the animals of Group X. The wall of this portion is thin, and within the tube is a mass of yolk material.

Observations were made on the development of certain parts of the skeletal apparatus. Neural arches of the vertebrae develop segmentally and hence reveal evidences for differences in the three groups of animals.

Larvae from Group A show a definite establishment of cartilaginous neural arches. The bases of these structures rest on the dorsolateral surfaces of the notochord, and extend dorsally and laterally around the neural tube (Fig. 7). The base of the arch is its widest and most developed portion. As the arch ascends there is a tapering off to a fine line of cells which bend medially to encircle the neural tube (Fig. 7). The tips of these arches have not as yet been embedded in their cartilaginous matrix. When viewed sagittally the bases of the neural arches appear as segmental blocks of cartilage, the more

anterior ones being the larger (Fig. 8). Cartilaginous blocks are present only in the anterior half of the embryo. In the posterior region, between the spinal ganglia there are masses of cells not as yet embedded in cartilage. This observation agrees with those from the cross section study in which the more posterior segments do not have well formed neural arch bases.

Group X larvae give a completely different picture. Cartilaginous arches are not present at any level of the antero-posterior axis. Elongate, spindle shaped cells are spreading out in the pathway that the neural arches take, but there is no gelatinous matrix (Fig. 9). The extent to which these cells have surrounded the neural tube is not as great as in the controls of Group A. They extend only half way up the sides from their basal positions on the dorso-lateral portion of the notochord. Examination of sagittal sections of these larvae gives much clearer evidence of the difference between neural arch formation in the larvae of Group X and the larvae of Group A. In the larvae of Group A the bases of the neural arches rest in between the well developed, segmentally arranged spinal ganglia. These are especially evident as well developed cartilaginous blocks in the region immediately back of the head. Lying in the myosepta are the dorsal elongations from the bases. Larvae of Group X examined from the sagittal aspect present no such picture. The spinal ganglia are present, but there are no segmental blocks of cartilage (Fig. 9).

Experimental animals of Group B have neural arches developed almost to the extent of those in the control larvae of Group A. Examination of cross sections shows small, thin neural arches extending part way up the sides of the neural tube (Fig. 10). The bases of these arches do not give evidence of being as well developed as those in the larvae of Group A, nor do the ascending arches extend around the neural tube. Thus, there is evidence of considerable development but it has not proceeded as far as in the controls. When viewed sagittally the bases of the arches are present in the segments immediately back of the head, appearing as blocks between the ganglia (Fig. 11). The antero-posterior extent of these arches is much less than in the controls, being limited to the first four or five segments.

Discussion and Conclusions

That the tempo of development and differentiation is accelerated in an amphibian when low temperature inhibited embryos are returned

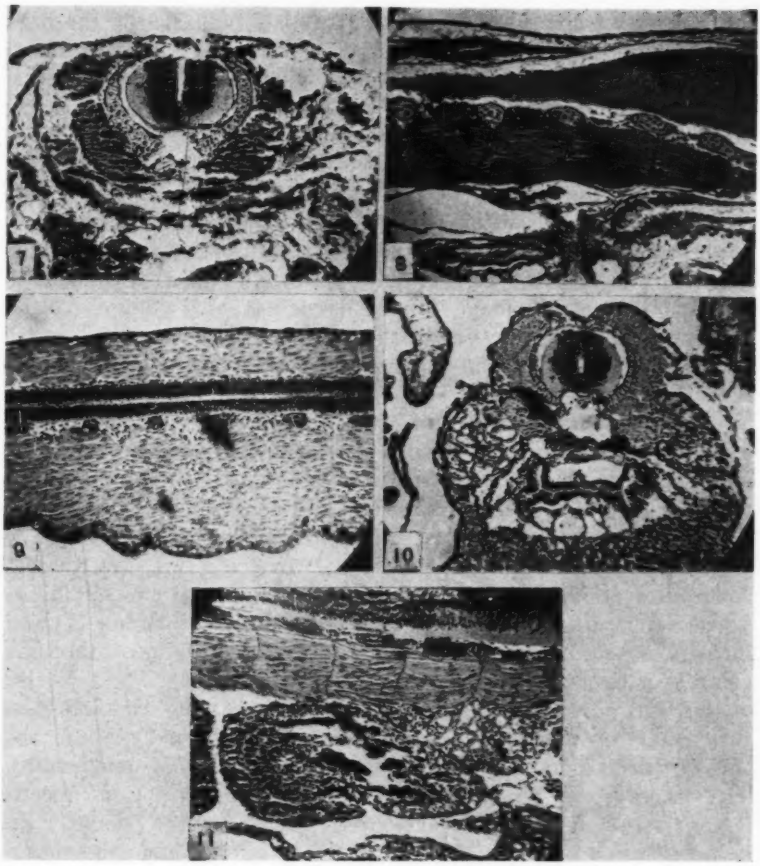

PLATE 2

Fig. 7. Neural arch bases and arches proper extending around neural tube in larva of Group A. These are highly cartilaginous and almost completely encircle the neural tube (X section).

Fig. 8. Neural arch bases appearing as segmented blocks of cartilage in control larva. Spinal ganglia lie in between arch bases (Sagittal section).

Fig. 9. Neural arch primordium in 72-hour younger larva of Group X. Mesenchymal cells are present between spinal ganglia in segmental arrangement, but no cartilage has been laid down (Tangential section).

Fig. 10. Neural arch bases and arches proper in cold treated larva of Group B. The cartilage is not as extensive as in controls nor are the arches as well developed (X section).

Fig. 11. Neural arch bases appearing as blocks in anterior region of cold-treated larva of Group B. Segmentally arranged cell masses appear between spinal ganglia; anterior cell masses embedded in cartilage matrix (Sagittal section).

to a higher temperature is evident. This is established by the work of the experimenters cited and by the results of these experiments.

It is necessary to point out that an individual flask contained larvae from the same egg mass, but not all flasks contained eggs from the same clutch. The eggs utilized in these experiments, therefore, were consistent in their sameness of age, but not in their source of egg mass. Nevertheless, the rates of development were almost completely uniform for the animals within a given lot, and were almost completely uniform lot for lot within the given groups. The histological picture observed and reported here is quite uniform in different animals of the same lot and in animals from different lots.

In an analysis of any one of the end points used in these experiments it is quite apparent that none of the three groups of animals is identical with another. The stomach of the animals of Group A is a well defined and well formed structure. It approximates the outline of a mature stomach. Larvae of Group B, the low temperature inhibited larvae have their stomachs somewhat elongate as do the controls, and there is a formation of a mucosal layer. From such evidence it can be stated that the inhibited organisms approximate in the development of their stomachs the control forms of Group A. This observation, however, is not clear evidence of post-inhibitory acceleration. In these experiments the animals of Group X were introduced and these were actually three days younger than the controls of Group A and the inhibited forms of Group B. By reason of this, 72 hours after the experiments had been initiated there were two groups of animals which were three days behind the controls. One of these groups was artificially retarded by the low temperature. The other group was actually three days younger. Consequently the analysis is of real significance by comparing Groups B and X. If no acceleration occurred the cold inhibited larvae should be similar to the larvae of Group X which were actually three days younger than the controls. If acceleration did occur in larvae of Group B there should be evidences of a greater amount of development in these larvae than in the larvae of Group X. The evidence from the study of the sectioned larvae of these two groups, B and X, showed a significant difference between the two. Development of the stomach and the intestine in the larvae which were actually 72 hours younger is obviously not as far advanced as in the forms which were retarded for 72 hours. The low-temperature inhibited organisms, with their

stomach and intestinal development more nearly approximating the controls of Group A, were obviously undergoing differentiation in this respect at a faster rate than the larvae of Group X.

The accelerated development is especially striking in metamerically arranged internal structures such as the mesonephros and the neural arches. These structures give evidence of more distinct changes and differences than can be gained from the examination of gross external features. If the larvae of one group show cartilaginous neural arch bases where those of another group do not there is obviously a sharp line of demarcation between the two. This is the case when comparing the animals of Group B with those of Group X. The significant and real differences between Groups B and X are the true demonstrations that low-temperature inhibited animals returned to control temperature develop at a faster rate than those not inhibited.

Buchanan's work ('38) on developmental acceleration showed that in several instances there were cold-inhibited larvae which completely overtook the control forms; a few even surpassed the controls. From the facts observed in these series of experiments no such degree of acceleration occurred.

It was evident without any doubt that in the beginning Groups A and B were chronologically identical. It was equally as evident that when the inhibited forms were removed from the low temperature to the higher temperature they were many stages behind those that had been developing at the higher temperature. The inhibited larvae, therefore, at the time of their removal to the higher temperature were still chronologically identical with those developing at the higher temperature, but morphologically unlike. They were so unlike one another that to one not knowing the previous history they would be declared as chronologically different. It is at this point that these experiments differ from any previous work done, by setting into the series eggs which were actually three days younger than the eggs of Groups A and B. As a result of placing these organisms into the experiments, the eggs of Group B (the low-temperature inhibited organisms) appeared as chronologically identical with the new series, Group X, rather than with the forms with which they were really chronologically identical. At the end of the experimental time, 450 hours later, the inhibited larvae of Group B no longer showed the vast differences between themselves and the control Group A. On the other hand, the inhibited eggs of Group B which initially

showed a seeming chronological sameness with the eggs of Group X, showed at the end of the experiments that they are really older than those of Group X and approximate the condition of those larvae of Group A with which they are chronologically identical. In order that this be effected, the tempo of development was of necessity greater in the low-temperature inhibited forms of Group B than in either of the other two groups, both of which were following a developmental rate characteristic of the temperature of 21°C.

It has been mentioned earlier in this paper that previous workers have demonstrated that the rate of development is rapid in early stages and there is a leveling off at later stages. Initial growth and differentiation is rapid up to a given time and then proceeds more slowly. Consequently, the almost complete overtaking of the controls by the low-temperature inhibited organisms of *Amblystoma punctatum* can be accounted for by two factors: (1) An initial acceleration of differentiative processes within all the primary systems necessary for larval life. In the earlier embryological development there are being laid down structures which constitute the primary framework of the organism. Such basic structures as brain, neural tube, somites, pronephric system, and vascular system are being formed so that the larva has an organization of parts sufficient to enable it to live as an integrated organism. (2) A less rapid deceleration of rate of development than in the controls due to a continuation of acceleration of differentiation of the secondary systems necessary for adult life. Such structures as mesonephros, neural arches, and a well developed digestive tract are examples of such secondarily necessary structures.

Summary

(1) Previous investigators have shown that amphibian eggs treated with low temperature and subsequently removed to a higher temperature have a more rapid development than do eggs developing constantly at the higher temperature. The criterion for this conclusion is based on observations of the animals stage-time relationships.

(2) The evidence from the present experiments shows that the differentiation of primary and secondary structures is proceeding at a more rapid rate in larvae which were previously treated with low temperature before being returned to higher temperature. This phenomenon is particularly evident in the secondary organ systems such as the stomach and intestine, the mesonephros and the neural arches.

(3) Particular emphasis should be placed on the continued rapid differentiation of the secondary structures to account for the almost complete overtaking of the controls by the low temperature inhibited organisms.

(4) Differentiation as well as growth is occurring at a more rapid rate in the amphibian larvae previously treated with low temperature.

References

ATLAS, MEYER. 1935. The effect of temperature on the development of *Rana pipiens*. *Phys. Zoöl.* **8**, No. 3.

BLISS, C. I. 1926. Temperature characteristics for prepuperal development in *Drosophila melanogaster*. *Journal Gen. Physiol.* **9**.

BODINE, J. H. 1925. Effect of temperature on the rate of embryonic development of certain Orthoptera. *J. Exp. Zoöl.* **42**.

BUCHANAN, J. W. 1938. Developmental acceleration following inhibition. *J. Exp. Zoöl.* **79**, No. 1.

———. 1940. Developmental rate and alternating temperature. *J. Exp. Zoöl.* **83**, 2.

CARPENTER, E. 1945. Effect of exposure to temperature of 5° to 10°C. on the survival and differentiation of embryonic thyroid tissue culture. *J. Exp. Zool.* **98**.

CLOWES, G. H., & KRAHL, M. E. 1934. Action of dinitro compounds on sea urchin eggs. *Science* **80**.

MORAN, T. 1925. The effect of low temperature on hen's eggs. *Proc. Roy. Soc. B.* **98**.

PARKER, J. R. 1930. Some effects of temperature and moisture upon *Melanoplus mexicanus mexicanus* Saussure and *Camnula pellucida* Scudder (Orthoptera). *Univ. Mont. Agric. Exp. Sta. Bull.*, No. 223.

PRICE, J. 1940. Time-temperature relations in the incubation of the whitefish, *Coregonus clupeaformis* (Mitchill). *J. Gen. Physiol.* **23**, No. 4.

RAPKINE, L. 1931. Sur les processus chimeques au cours de la division. *Ann. de Physiol. et de Phys.-Chem. Biol.* **7**.

RYAN, F. J. 1941. The time-temperature relation of different stages of development. *J. Exp. Zoöl.* **81**.

———. 1941. Developmental rate and structural expression. *J. Exp. Zoöl.* **88**.

STOCKARD, C. R. 1921. Developmental rate and structural expression. *Amer. Jour. Anat.* **28**.

TYLER, A. 1942. Developmental processes and energetics. *Quart. Rev. of Biol.* **17**, Nos. 3 and 4.

TYLER, A., & SCHEER. 1937. Inhibition of fertilization in eggs of marine animals by means of acid. *J. Exp. Zoöl.* **75**.

WILLIAMS, C. M. 1946. Physiology of insect diapause; The rôle of the brain in the production and termination of pupal dormancy in the giant silkworm, *Platysamia cecropia*. *Biol. Bull.* **90**, No. 3.

Growth, 1950, **14**, 341-351.

THE ROLE OF pH AND BUFFERING CAPACITY OF THE MEDIUM IN BACTERIAL GROWTH (BACTERIMETRIC STUDIES VI)*

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We have previously (1) proposed a single basal medium for the determination of folacin and related compounds with the two organisms, *Lactobacillus casei* 7469 and *Streptococcus lactis R* 8043, which have been used for this purpose under separate conditions since 1945 (2). The growth responses obtained with these and other bacteria are usually well reproducible within each experiment but subject to substantial day-to-day variations despite procedural standardization. Elimination of these variations and an understanding of their causes is desirable on theoretical and practical grounds. The present study will show that the pH and the buffering capacity of the medium can be important determinants of quantitative growth response.

EXPERIMENTAL

Medium—The previously described medium (1) has been further modified in details, and ascorbic acid (3) has been added. It now contains, per cc. of finished medium, the following:

20.5 mg. (0.25 mM) of sodium acetate,
20.0 mg. of glucose,
6.25 mg. of hydrolized casein (0.125 cc. of Casein Hydrolysate Enzymatic, Nutritional Biochemicals Corporation, Cleveland 5, Ohio),
1 *N* NaOH as required for pH adjustment,
3.48 mg. (0.020 mM) of K_2HPO_4,
3.40 mg. (0.025 mM) of KH_2PO_4,
0.25 mg. of ascorbic acid,

*Aided by a research grant from Sidney Scott and an institutional grant from the American Cancer Society.

0.20 mg. each of $MgSO_4 7H_2O$, L-cystine and DL-alanine,
0.10 mg. of L-asparagin,
10 γ each of adenine, guanine, uracil, xanthine, $FeSO_4.7H_2O$, $MnSO_4.H_2O$, and NaCl,
0.50 γ each of nicotinic acid, calcium pantothenate, riboflavin, and thiamin hydrochloride,
0.20 γ of pyridoxamine hydrochloride,
0.10 γ of p-aminobenzoic acid,
4.0 mγ of biotin.

Titration Curves of Media—To evaluate the buffering capacity of the medium and its components, buffering curves were obtained by electrometric titrations with 1.00 *N* NaOH in 1 cc. intervals. The volume at the beginning of the titrations was 80 cc. which contained the ingredients, except NaOH, corresponding to 100 cc. of basal, i.e., 200 cc. of finished, medium. Figure 1 shows the results obtained with three different media and the corresponding lots of the commercial hydrolysate. As a check on the accuracy of the measurements the acid-base requirements of the non-hydrolysate ingredients of the medium were calculated from the differences between the titration curves of the medium and the hydrolysate. The resulting titration curve of the ingredients, essentially identical in the three cases, is shown by a separate curve on the figure.

Media of Different pH Values—For this work medium M 2 of Fig. 1 was used. Twelve separate portions of the basal medium were prepared, each adjusted with NaOH to a different acidity between pH 6 and 7. The actual pH values were determined and redetermined 24 hours later. The average differences between pairs of readings was +0.01. Three cc. portions of each medium were combined with 3 cc. portions of folacin solution, obtained by dilution of a $10^{-5}M$ stock solution of folacin in $10^{-2}M$ NaOH, to yield a folacin concentration of $1.25 \times 10^{-10}M$ for the series to be inoculated with L. casei and $2.50 \times 10^{-10}M$ for the S. lactis series.

Inocula—The medium used for the growth of inocula had the same general composition as that used for the experiment proper. However, its pH was 6.50 and it contained $1.5 \times 10^{-10}M$ folacin for L. casei and $3 \times 10^{-10}M$ folacin for S. lactis. Six cc. cultures in these two media were grown at 37.8° and arrested by immersing the tubes in ice water when an AOD (adjusted optical density, cf. (4))

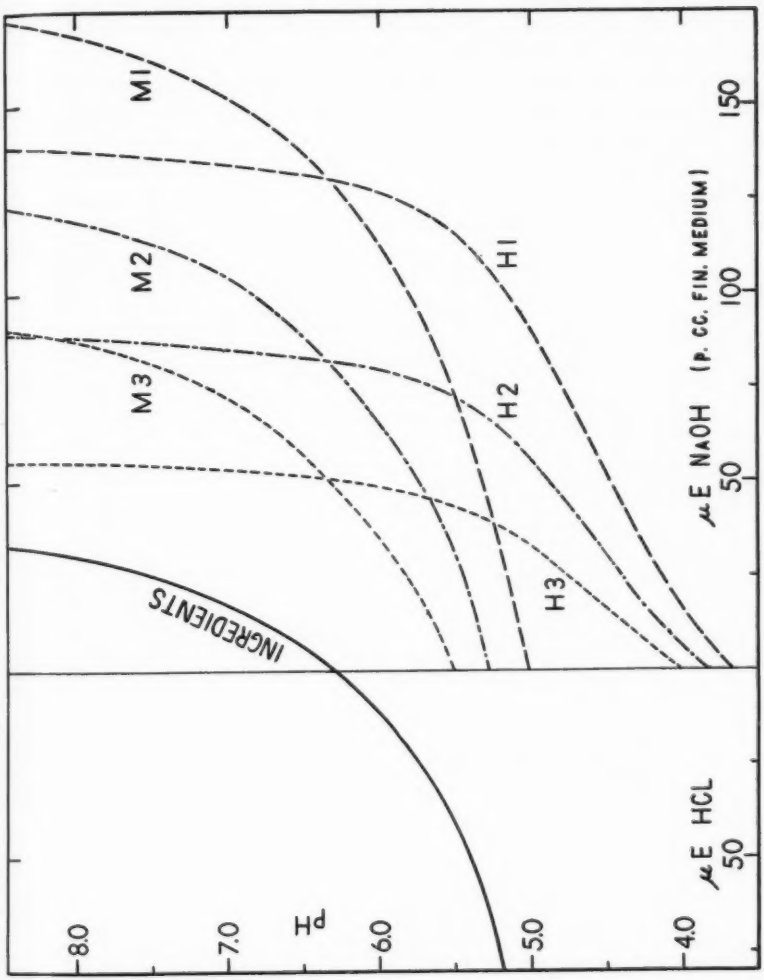

FIGURE 1

Titration curves of media and components. Each medium has the composition shown in the text under "Medium". Titration curves of three media (M1, M2, M3) and the corresponding lots of commercial hydrolysate used (H1, H2, H3) are shown, together with the titration curve of the sum of the ingredients added to a hydrolysate to make a basal medium.

of 100-120 had been reached. Several regrowths of these cultures in the same medium by using 0.25 cc. of the previous culture as the inoculum yielded the experimental inocula. Of these cultures which are chilled at AOD 120, in the midst of their logarithmic growth phase, one drop is used for each experimental tube, so that the calculated initial optical density is 1. With inocula of this type the ensuing growth shows no lag phase.

Growth in Media of Different pH—Two tubes of each pH level and the appropriate folacin content were inoculated with each organism

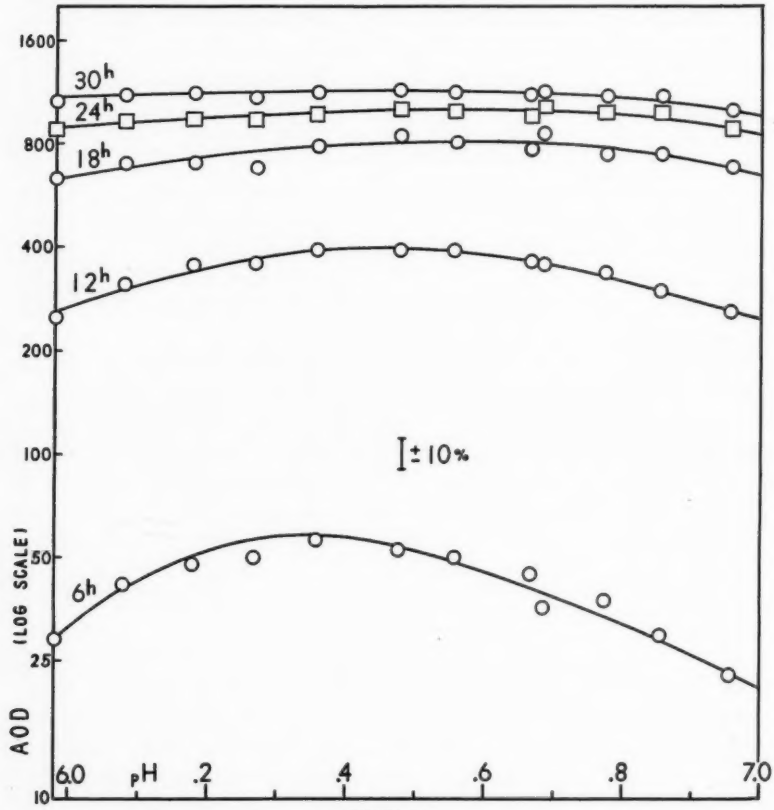

FIGURE 2
Growth of *Lactobacillus casei* at different pH levels.

and incubated at 37.8°. Spectrophotometric turbidity readings at 675 mμ were taken at frequent intervals for 48 hours. Equipment and techniques have been previously described (5). For each organism one tube of each pH level was removed and chilled when growth had reached the level of AOD 400. This series of tubes was used for pH determinations while the other member of each pair was continued for growth curve readings to the end of the experiment.

The experiment yielded 24 individual growth curves, together with pH readings at the beginning, after AOD 400 had been reached, and

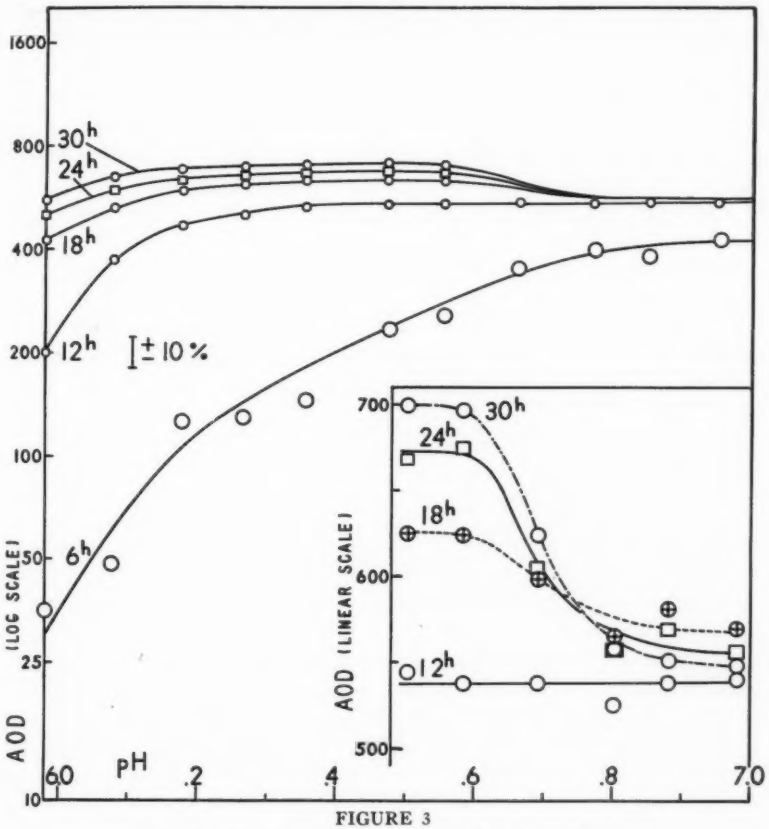

FIGURE 3

Growth of *Streptococcus lactis* at different pH levels.

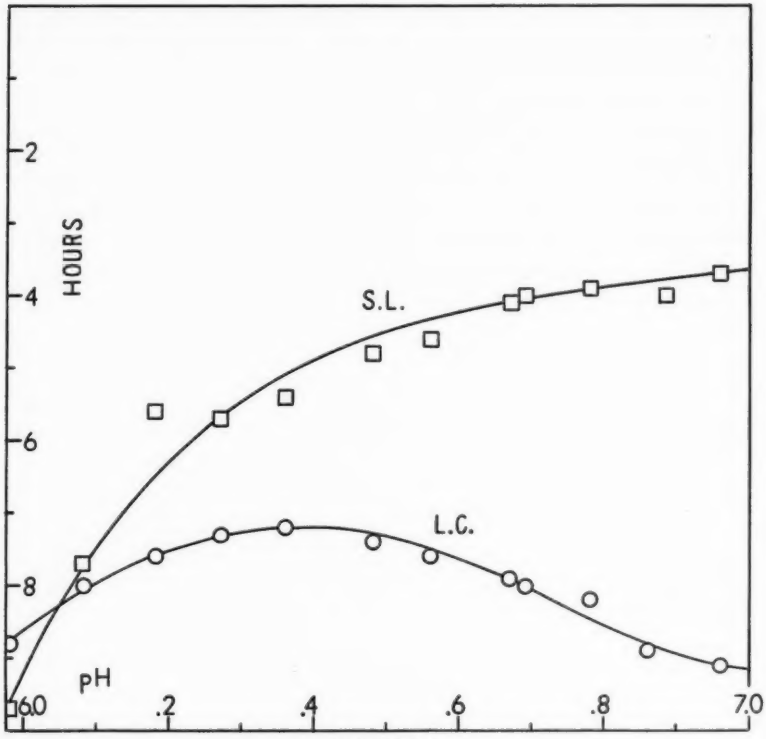

FIGURE 4
Early growth of the two organisms at different pH levels.

at the end of the experiment. The resulting information is presented in Figures 2-5. It is apparent that initial growth rates vary greatly and that L. casei grows fastest near pH 6.35 while for S. lactis the optimum is at pH 7.0 or beyond. However, in the later stages of growth the point of maximum response shifts somewhat to the less acid side for L. casei and to the more acid side for S. lactis. As shown in detail by the inset of Figure 3, the measurements indicate slow lysis of S. lactis during the terminal stages of the experiment in tubes of initial pH values of 6.7 or higher. This phenomenon, as well as the general trend of the results shown in Figures 2 and 3, has been confirmed by several exploratory experiments which preceded those recorded.

To further illustrate the nature of early growth, Figure 4 shows the periods of time, read from the experimental curves, required to reach AOD 100 at different pH levels. The resulting curves repeat the trends shown by the 6-hour curves of Figures 2 and 3.

Acid Formation—The initial pH values, as shown in the figures, ranged from 5.98 to 6.96. When AOD 400 had been reached pH ranged from 5.69 to 6.36 for L. casei and from 5.80 to 6.52 for S. lactis. After 48 hours of incubation pH and corresponding AOD ranged from 4.99 and 1290 to 5.28 and 1065 for L. casei, and from 5.48 and 646 to 5.59 and 506 for S. lactis.

From the pH values corresponding to AOD 400 the amount of acid formed at this stage can be calculated with the aid of the titration curve of medium M 2, shown in Figure 1. The results are given in Figure 5. They indicate that acid formation accompanying a given amount of bacterial growth as measured by the resulting turbidity, is at a minimum for both organisms when the initial pH is 6.3. It further appears that acid formation per unit growth, as defined, is twice as large for L. casei as for S. lactis. Although the values given on the ordinate of Figure 5 can be considered as relative only, since they are calculated in terms of strong acid while weak acid is formed in bacterial metabolism, the difference seems worth noting since both organisms are reported to be homofermentative Lactobacteriaceae (6) which produce acid by quantitative conversion of sugar to lactic acid.

Growth Response and pH of Inoculum Medium—Because of the marked differences in growth rate and net response resulting from differences in the pH of the medium the possible effect of the pH at which the inoculum is grown, on subsequent response was also examined. The results of this experiment are summarized in Table 1. The data indicate that the effect of variations in the pH of the inoculum medium, if any, is small compared with the effect of the pH of the medium being inoculated.

Buffering Capacity of Different Media—As a part of this study the buffering capacities of a few different types of bacterimetric media were compared by electrometric titrations. The findings, summarized in Table 2, show the range of the changes which may result from variations in buffer content.

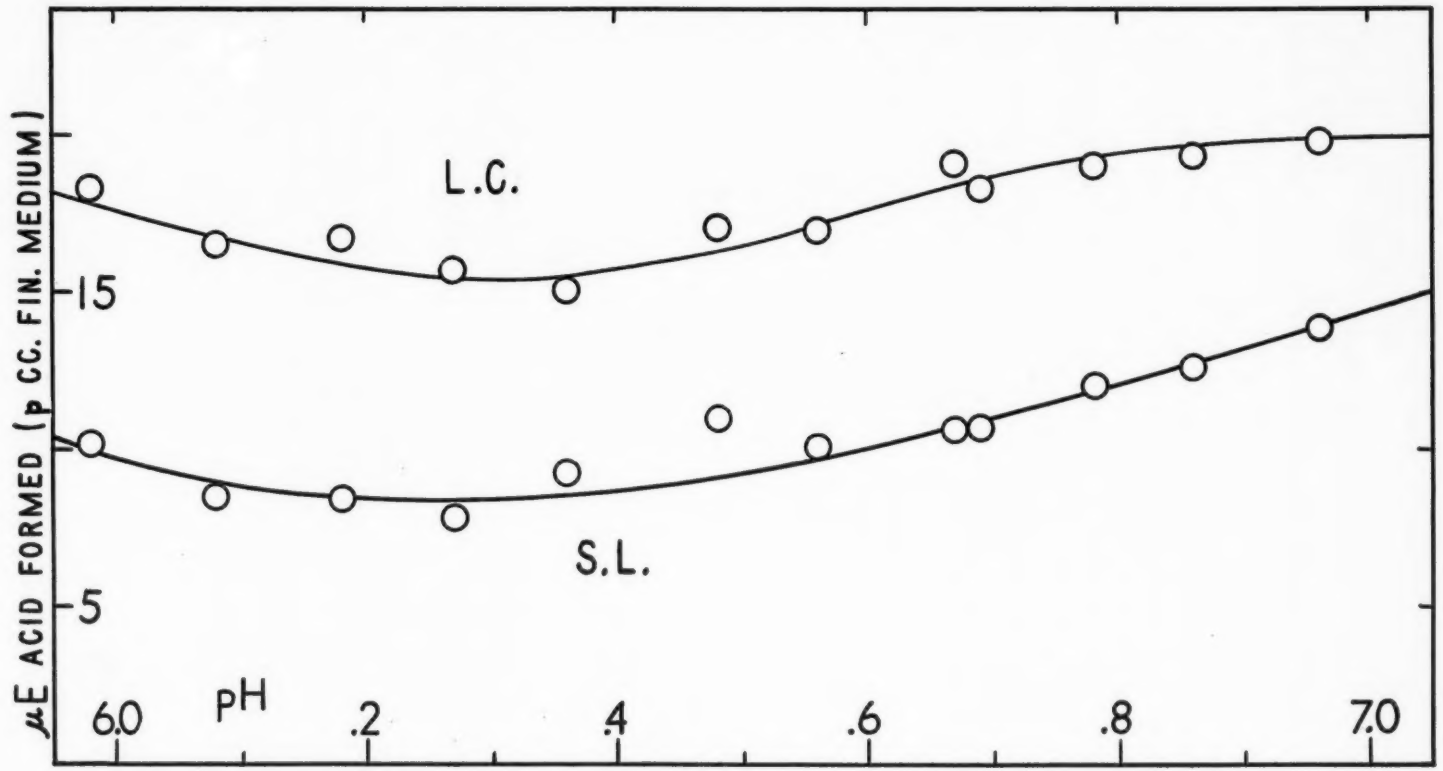

FIGURE 5

Amount of acid liberated at equal turbidity levels by the two organisms when growing in media of different pH.

TABLE 1
GROWTH RESPONSES AND pH OF INOCULUM MEDIUM

Inoculum grown at	at pH 6.14		at pH 6.42		at pH 6.64	
	17h	24h	17h	24h	17h	24h
L. casei response						
pH 6.14	600±8	879± 7	684± 9	943±12	729±16	997±18
pH 6.64	601±7	870± 8	679±10	936± 9	721± 4	950±15
S. lactis response						
pH 6.14	590±6	753±12	567± 7	606±18	524± 6	513± 7
pH 6.64	600±6	759± 9	540± 5	559± 4	512± 9	497± 8

Inocula (L. casei 1.5 x $10^{-10}M$ folacin, S. lactis 3 x $10^{-10}M$ folacin) were repeatedly grown to AOD 100. All responses (L. casei, to 1.25 x $10^{-10}M$ folacin; S. lactis, to 2.5 x $10^{-10}M$ folacin) represent the average of four tubes, with average deviations; all expressed in AOD units.

TABLE 2
BUFFERING CAPACITY OF DIFFERENT MEDIA

pH	μE base (NaOH) required to change pH of 1 cc. finished medium from pH 6.50 to pH indicated		
	Medium A	Medium B	Medium C
6.0	−8.7	−23	−73
6.25	−3.5	−11	−40
6.50	0.0	0	0
6.75	+2.2	+ 8	+39
7.00	+3.6	+15	+70

Medium A: Amino acid medium of Stokes (7) with sodium acetate content increased 3.33 fold, to 0.25 *M*.
Medium B: This is medium M 2 of Figure 1.
Medium C: Our modification (8) of Stokes medium: 0.30 *M* phosphate of pH 6.5 and 0.075 *M* acetate.

DISCUSSION

A study of recent review articles on microbiological analytical methods shows that acidity and buffering capacity of the medium as growth determining factors have received little detailed attention. In practical methods adjustment of the medium to pH 6.8-7.0 with concentrated alkali and with the aid of an external indicator is a typical recommendation. The present study shows that under the experimental situations considered pH is an important determinant of bacterial growth, thus suggesting that precise control of this factor can materially contribute to the standardization of analytically used bacterial responses.

It is well recognized that avoidance of bacteriostatic acidity levels resulting from metabolic acid liberation requires adequate buffering

capacity of the medium. The present results permit the further conclusion that moderate changes in the buffering capacity of the medium such as may result from the buffering power of the added analytical specimen, may significantly affect growth response. Certain cases of "drift" in the values obtained at different assay levels may be due to this factor rather than to the action of undefined inhibitory or stimulatory factors. From the points of view of the present and preceding observations media of high buffering capacity offer definite advantages: they minimize pH changes resulting from growth itself, and thus increase the range of linearity of response (8), and they minimize possible changes in buffering capacity introduced by the analytical specimen, thereby eliminating a possible cause of "drifts".

Summary

1. The buffering capacity of enzymatic casein hydrolysates and of bacterial media consisting of these hydrolysates combined with pure chemicals has been determined.

2. The effect of variations of pH in the range of pH 6 to pH 7 on the growth of *Lactobacillus casei* 7569 and *Streptococcus lactis R* 8043 in a medium containing enzymatic casein hydrolysate, with folacin as the limiting nutrient, has been studied. L. casei showed a growth rate maximum at an initial pH of 6.35 while S. lactis grew fastest at pH 7.0 or beyond.

3. The amount of acid formation associated with a given amount of growth, measured turbidimetrically, has also been determined for both organisms. Relative acid formation was found to be lowest for both organisms at an initial pH of approximately 6.3, and to be approximately twice as high for L. casei as for S. lactis.

4. Inocula were grown in the same kind of medium as was used for the experimental growths. The inocula were employed while in their logarithmic phase, and no lag phase was encountered in the ensuing growth under these conditions.

5. Some orientative comparisons of the buffering capacity of media of different type and buffer content were made.

6. The findings have been discussed in terms of their practical significance.

References

1. Toennies, G., & Gallant, D. L. 1949. Bacterimetric Studies III. Blood level studies on teropterin metabolism. *J. Lab. Clin. Med.*, **34**, 501.
2. Teply, L. J., & Elvehjem, C. A. 1945. The titrimetric determination of "Lactobacillus casei factor" and "folic acid". *J. Biol. Chem.*, **157**, 303.
3. Rickes, E. L., Koch, P. J., & Wood, T. R. 1949. Additional observations on the rate of growth of Lactobacillus casei. *J. Biol. Chem.*, **178**, 103.
4. Toennies, G., & Gallant, D. L. 1949. The relation between photometric turbidity and bacterial concentration (Bacterimetric Studies IV). *Growth*, **13**, 7.
5. ———. 1948. Bacterimetric Studies I. Factors affecting the precision of bacterial growth responses and their measurement. *J. Biol. Chem.*, **174**, 451.
6. Breed, R. S., Murray, E. G. D., & Hitchens, A. P. 1948. Bergey's Manual of Determinative Bacteriology. Baltimore: Williams and Wilkins. Pp. 1529.
7. Stokes, J. L., Gunness, M., Dwyer, I. M., & Caswell, M. 1945. Microbiological methods for the determination of amino acids. II. A uniform assay of the ten essential amino acids. *J. Biol. Chem.*, **160**, 35.
8. Toennies, G., & Gallant, D. L. 1949. Individual characteristics of the bacterial growth responses induced by different amino acids. (Bacterimetric Studies V.) *Growth*, **13**, 21.

Growth, 1950, **14**, 353-356.

"CELL GROWTH AND CELL FUNCTION, A CYTOCHEMICAL STUDY"

TORBJÖRN CASPERSSON
W. W. Norton and Co., New York, 1950

A REVIEW BY N. B. KURNICK

The importance of Dr. Caspersson's recognition of the possibility of applying the ultraviolet microscope as a chemical analytical tool is now universally recognized. His development of a microspectrophotometer and its application to the problems of cell biology, particularly the rôle of nucleoproteins in cellular reproduction and function, are well known. These contributions have greatly extended our knowledge of the chemical structure of cells and produced fruitful hypotheses of the chemical physiology of growth processes. It is therefore a great pleasure to have compiled in a single volume the results of the investigations which have come from Dr. Caspersson and his collaborators. His new book, "Cell Growth and Cell Function," based on his Salmon Memorial Lectures, is such a compendium of the work of the Institute for Cell Research in Stockholm, including a bibliography of all contributions from that institution. His book is addressed to workers in the fields of medicine, neurology and psychiatry, biological physics and biological chemistry, and genetics.

Although valuable for an interpretation of the results cited in the later chapters, Chapters 2 and 3 contain material which is apt to be somewhat obtuse for workers in most biological fields other than those directly concerned with physical optics. In these chapters Caspersson considers the apparatus for quantitative microspectrophotometric measurements, the optical requirements, and the limitations imposed thereby. The theoretical discussion is brief, and is illustrated with very clear diagrams. However, such curves as Figure 16 might have been provided with a more illuminating description for workers lacking experience in physical optics. Limitations as to the size of the object and the effects of refraction and light dispersion are briefly considered. Here a slip has entered which appears to have resulted from an artist's error in the preparation of a figure

for Professor Caspersson's original thesis. The minimum diameter of a particle suitable for photometric analysis is calculated to be three to four times the wave-length of the incident light. This value is given as approximately 200 $\mu\mu$ instead of 2,000 mμ or 2 μ (using green incident light). The relative merits of the photographic and photometric methods are briefly considered, with the conclusion that the former excels in simplicity and the latter in accuracy. The apparatus designed by Caspersson for microspectrophotometry is described in some detail both in the text and in the figures. The importance of proper tissue preparation and embedding and the use of high aperture lenses is stressed. The changes in light dispersion and refraction incident to death and fixation are well presented and due stress is placed on the importance of complete spectral analysis for the proper evaluation of these changes. Chapter 3 deals with the interpretation of the microspectrophotometric curves obtained. The theoretical background is only sketchily covered, reference being made to the original papers. A simple formula for the determination of protein and nucleotide content based on two wave lengths is presented. This is really only of relative value, however, since the effect of light loss due to dispersion is neglected. This, unfortunately, can be a significant effect in many materials. In this chapter, Caspersson also refers to the X-ray spectrographic procedures developed by Engström, which are of principal value in the determination of mass and of some of the heavier elements only rarely encountered in tissues.

In Chapters 4 and 5, Caspersson presents the theory of the mechanism of protein synthesis in the cell during mitosis and during the resting phase, as originally proposed by Caspersson and Schultz. Caspersson finds that the nucleic acid content of the chromosome increases during prophase. During the course of this increase in nucleic acid, he reports also a conspicuous decrease in the amount of protein. The first observation is generally concurred in by other authors. However, the decrease in protein has been questioned, and it has been claimed by others that the increase in nucleic acid during prophase is associated with a reduplication of all chromosomal material, including protein. It is quite possible that the spectrophotometric method is unreliable for the measurement of protein in the face of considerable amounts of nucleic acid, which tend to obscure the much lower absorption coefficients of protein. A brief paragraph and a figure refer to the effects of digestion with proteolytic enzymes on chro-

mosomes in grasshopper meiosis. The description is unfortunately all too brief for proper interpretation of the accompanying figure. In his description of telophase, Caspersson describes the swelling of the chromosomes which gradually fill the spaces between them, resulting in the rounded homogeneous nucleus of interphase which is now the generally accepted interpretation. However, in his discussion of the interphase nucleus, he considers the fixed appearance of the nucleus of Drosophila salivary glands. These show the familiar ribbon-like chromosomes with well-marked bands. This appears to be the same type of condensation artifact as the well-demarcated chromatin of interphase nuclei of other materials following fixation. Since the living nucleus of the Drosophila salivary, like that fixed directly in neutral formulin by dissecting the living larvae in that fixative, appears to be a homogeneous vesicle in which the chromosomes are in contact with one another and are fully swollen with no bands or interbands, the analysis of band and interband areas may be of rather limited significance. The finding of bands rich in nucleic acid interspersed with nucleic acid-free interband spaces, is probably simply the result of the shrinkage of the nucleo-protein substance of the chromosome into compartments leaving relatively empty spaces between them. It is somewhat disturbing to note, too, that the measured area in the salivary chromosome indicated in Figure 40 measures approximately 200 mμ in diameter, thus failing to satisfy the criterion for size required by the theoretical analysis presented earlier. A theory is presented which assigns distinct rôles to the euchromatic and heterochromatic regions of the chromosomes. The former are thought to produce proteins rich in tyrosine and tryptophane while the heterochomatic regions produce proteins rich in diamino acids which collect in the nucleolus. Here they are thought to be stored in association with some ribonucleic acid until required for the direction of cytoplasmic protein synthesis. This material subsequently diffuses from the nucleolus across the nucleus into the cytoplasm, where it stimulates the synthesis of ribonucleoprotein at the nuclear membrane. Whereas the significance of cytoplasmic ribonucleoprotein in the synthesis of cytoplasmic protein and of proteins elaborated by the cytoplasm for secretory purpose is generally accepted, this train of events remains largely hypothetical. A number of examples are presented in this chapter of the correlation of ribonucleoprotein synthesis in the cytoplasm with cell growth or cell function. In Chapter 6 some

abnormal examples of this association are presented. These are taken primarily from tumor material, in which it is found that the actively growing tumor cells, particularly those placed advantageously in regard to nutrition, are found to be rich in cytoplasmic and nucleolar ribonucleoprotein.

Chapter 7 is devoted to the consideration of protein synthesis under the direction of ribonucleic acid in lower forms of life such as yeasts, bacteria, and viruses. In the yeast, again, the special position of heterochromatin as the governing portion of the chromosome in protein synthesis is presented. It is suggested that the heterochromatin of yeast contains genes which, unlike those of the euchromatin, are unequally distributed at division and may vary in amount from tissue to tissue. In the bacteria, as in the higher forms, the ribonucleoprotein content is found to be directly proportional to the rate of protein synthesis. The viruses are found to stimulate, at least at first, the system for protein synthesis of the parasitized host cell, but the synthetic mechanism is directed toward the production of virus protein. The simplest viruses, which contain only ribose nucleotides, are thought to consist of only one protein group, the synthesis of which is directed by the ribonucleic acid. The higher types of viruses, which are assumed to contain several different kinds of proteins that are simultaneously reproduced, contain a mechanism resembling that of the higher forms for this process and therefore are found to contain some desoxyribose nucleotides. The problem of plastids has, perhaps wisely, been entirely omitted.

Chapter 8 is a general summary of the theory of protein synthesis by the organism, and contains reproductions of some of the figures presented in earlier chapters, describing this mechanism.

Although there is no index, the table of contents includes subheadings covering the material presented in most of the chapters. The format of the book and the printing are remarkably good. The text is almost free of misprints. We found only two minor typographical errors, each of them in the text to figures. In the text to Figure 23B, the descriptions of curves 4 and 5 are reversed.

In general, the book is well-written and very readable, and is a welcome contribution from a pioneer in the field of quantitative cytochemistry. We shall look forward to further important contributions from the author and his Institute.